



Phytoremediation potential for poly- and perfluoroalkyl substances (PFASs) using various plant species

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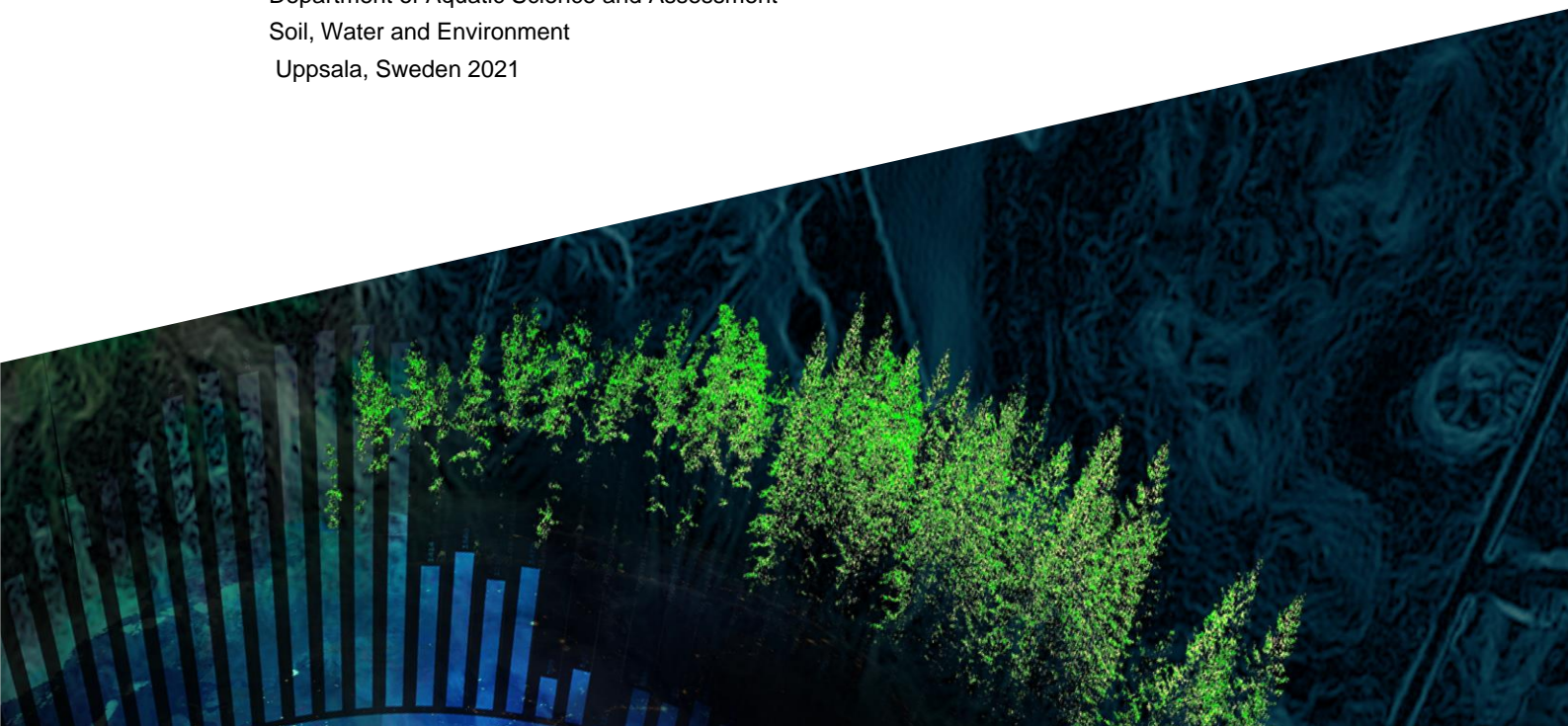
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Abstract

Poly- and perfluoroalkyl substances (PFASs) have unique chemical characteristics and are used in a wide variety of products. PFASs have been found in wild animals and humans and have shown adverse effects. Different remediation methods have been developed to remediate PFASs in the environment. Phytoremediation is one of the remediation methods with certain advantages, such as being low-cost, energy-efficient, less harmful, flexible and effective in removing PFAS. This study aims to evaluate the potential of plant uptake of 12 different PFAS from mustard (*Brassica juncea*), sunflower (*Helianthus annuus*) and industrial hemp (*Cannabis sativa*). In addition, the effect of amendments i.e., fertilizers, microbes, and the combination of fertilizers with microbes on the uptake of PFASs was evaluated. Pot experiments were performed in a greenhouse at SLU. Four different treatments with different amendments (fertilizer and microbial fertilizer) were applied to the plants in this experiment. The results indicated that PFASs were mostly transported and accumulated in the leaves, as opposed to the other plant compartments. Hemp had the highest levels of PFASs in the plant tissue (14.3 µg/plant) in comparison to sunflower (12.9 µg/plant) and mustard (8.3 µg/plant) in all control samples. Even though the total uptake of PFASs by mustard is the lowest, the PFAS concentration in mustard leaf is the highest (1.2 µg/g dry weight (dw)) among all plant compartments. The amendment with nutrient fertilizer and the amendment with microbe fertilizer decreased the PFAS concentration in the plant tissue, due to the sorption between PFASs in the soil and the fertilizer added. In conclusion, hemp seems a promising candidate for phytoremediation of PFAS contaminated soil.

Keywords: Phytoremediation, PFAS, mustard, sunflower, industrial hemp.

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Abbreviations

AFFF	Aqueous film-forming foam
BCF	Bioconcentration factor
ECF	Electrochemical fluorination process
K _{ow}	Octanol-water partition coefficient
PFAAs	Perfluoroalkyl acids
PFASs	Per- and Polyfluoroalkyl substances
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutane sulfonic acids
PFC	Per- and polyfluorinated chemical
PFCAs	Perfluoroalkyl carboxylic acids
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHpA	Perfluoroheptane acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acids
PFPeA	Perfluoropentanoic acid
PFSAs	Perfluoroalkyl sulfonic acids
PFUnDA	Perfluoroundecanoic acid
RCF	Root concentration factor
SCF	Shoot concentration factor
SOC	Soil organic carbon
SOM	Soil organic matter
SPE	Solid-phase extraction
TF	Translocation factor
UNEP	United Nations Environment Programme
UHPLC-MS/MS	Ultrahigh performance liquid chromatography tandem mass spectrometry

1. Introduction

Poly- and perfluoroalkyl substances (PFASs) have been used widely in our daily life and have been detected in different environmental compartments (Butt et al., 2010; Banzhaf et al., 2016; Arinaitwe et al., 2021). Their bioaccumulation ability in living organisms also attracted the attention from the scientists (Martin et al., 2003a; Martin et al., 2003b; Ahrens et al., 2016; Giesy and Kannan., 2001). The pathways of human exposure to PFASs include drinking water (Banzhaf et al., 2016; Boiteux et al., 2012), food (Clarke et al., 2010), air, and dust (Ericson Jogsten et al., 2012). Accordingly, a series of studies conducted on PFASs have revealed that PFASs were detected in the human body and have adverse health effects (Ait Bamai et al., 2020; Alexander et al., 2003; CDC, 2017; Cui et al., 2020; Kung et al., 2020).

Different environmental remediation methods have been developed, proven and tested for removing PFASs in contaminated soil including physical, chemical and biological remediation methods (Ahmed et al., 2020; Liu et al., 2013; Lu et al., 2020). Phytoremediation is one of the biological methods for removing toxic substances from the environment which has several advantages over physical and chemical remediation methods (Mahinroosta and Senevirathna, 2020). Phytoremediation of PFASs has been investigated and discussed whether it is a better solution for remediating PFASs in many kinds of plants (Ahmad et al., 2016; Bizkarguenaga et al., 2016; Blaine et al., 2013; Felizeter et al., 2012; Felizeter et al., 2014; Gobelius et al., 2017; Krippner et al., 2014; Lasee et al., 2020; Stahl et al., 2009; Wen et al., 2014; Xiang et al., 2018). Only a few studies have examined how different combinations of fertilizer and microbial fertilizer affect the uptake of PFASs by plants, which will be studied in this paper.

1.1. Aim and hypotheses

The aim of this study was to examine the phytoextraction efficacy of PFASs by three different plant species (i.e., mustard (*Brassica juncea*), sunflower (*Helianthus annuus*) and industrial hemp (*Cannabis sativa*)) as a viable PFAS remediation method. The objectives are to:

- Assess the uptake and distribution of PFASs by hemp, sunflower and mustard in different tissue compartments.
- Evaluate the effect of amendments i.e., fertilizers, microbes and the combination of fertilizers with microbes on PFAS uptake.
- Assess the concentration factors and make correlations with PFAS carbon chain length and functional groups.
- Estimate the time of using plants to perform phytoremediation under real conditions.

The following hypotheses were examined in this experiment:

- Hemp and sunflower will take up more PFASs in comparison to mustard due to their higher growth rate
- Short-chain PFASs can be efficiently removed by phytoremediation compared to long-chain PFASs.
- The PFAS uptake will increase with fertilizer and/or microbe fertilizer addition.
- Short-chain PFASs will be more concentrated in the shoots and long-chain PFASs will be more distributed in the roots.

2. Background

2.1. PFASs

2.1.1. What are PFASs

Per- and Polyfluoroalkyl substances (PFASs) are a group of aliphatic substances that have been largely utilized in various products, i.e., adhesives, food packaging, heat-resistant non-stick cooking surfaces and firefighting foams (CDC, 2017). PFASs were introduced to the world by humans around 1950 (Kissa, 2001; Buck et al., 2011; Zhao et al 2016). The chemical structure of perfluoroalkyl substances is mainly aliphatic substances, where all H atoms on the carbon chain are replaced by F atoms. However, the H atoms on the functional groups are not included in this. On the other hand, if some but not all of the H atoms on the carbon chain are replaced by F atoms, and the other conditions remain the same, these substances are referred to as polyfluoroalkyl substances (Buck et al., 2011)

The structure of PFAS chemicals consists of two components, an oil-soluble component and a water-soluble component. The water-soluble part is the functional group, whereas the oil-soluble part is the fluorinated carbon chain (Buck et al., 2011). This characteristic makes PFASs a perfect surfactant with a high surface activity compared to other hydrocarbon analogs (LehmLer, 2005).

The carbon-fluorine bond (C-F bond) is the most important part of PFAS structures, as it is the strongest bond in organic chemistry (O'Hagan, 2008). The fluorine ion possesses highest electronegativity (close to 4) among all the elements (Ryss, 1960). The bond dissociation energy for the C-F bond is the highest among all other covalent bonds, which is 105.4 kcal mol⁻¹. Furthermore, the C-F bond length is the shortest in all carbon-halogen bonds (O'Hagan, 2008). Considering the reasons outlined above, PFASs are highly stable and persistent (Conder et al., 2008; Cousins et al., 2020).

Perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) belong to the perfluoroalkyl acids (PFAAs) and have the general chemical formula $C_nF_{2n+1}COOH$ and $C_nF_{2n+1}SO_3H$, respectively (Buck et al., 2011). PFAAs are important because they were widely used in industrial processes and daily necessities. Another reason is that these chemical substances are often released directly or indirectly into the environment as degradation byproducts of the precursor substances, i.e., perfluoroalkane sulfonyl fluorides (PASFs; $C_nF_{2n+1}SO_2-R$) and n:2 fluorotelomer raw materials ($C_nF_{2n+1}CH_2CH_2-R$) (Wang et al., 2015), which make PFAS pollution more difficult to deal with (Buck et al., 2011). PFASs are classified as either long-chain and short-chain. Long-chain PFASs (including PFSAs ($C_nF_{2n+1}SO_3H$, $n \geq 6$) and PFCAs ($C_nF_{2n+1}COOH$, $n \geq 7$)) have a hydrophilic tendency. On the other hand, short-chain PFASs (including PFSAs ($C_nF_{2n+1}SO_3H$, $4 \leq n < 6$) and PFCAs ($C_nF_{2n+1}COOH$, $3 \leq n < 7$)) have a water-soluble tendency (Buck et al., 2011).

Octanol-water partition coefficient (K_{ow}) is a factor to determine whether chemicals have a hydrophilic or lipophilic property. Zhang et al., (2019) found out that short-chain PFAAs have a

relatively small K_{ow} compared to long-chain PFAAs, which means that short-chain PFAAs are more hydrophilic and long-chain PFAAs are more lipophilic.

2.1.2. The distribution for PFASs in our environment

The 3M company began producing PFASs in 1945 using the electrochemical fluorination process (ECF) (3M, 1999). The amount of PFASs used increased in the years following this time. The first formal regulation of this type of chemical substance, the Stockholm Convention, went into effect in 2009 and prompted the public to pay attention to PFAS (Overview on PFOS, its salts and PFOSF, 2009).

PFASs are widely distributed around the world. In Europe, Ahrens et al., (2010) detected low PFAS concentration in the North Sea, the Baltic Sea, and the Norwegian Sea, mainly caused by human activities. Arinaitwe et al., (2021) detected low concentrations of PFASs in the river and urban discharge in Africa. The presence of PFASs in the Arctic can also be attributed to atmospheric transport of precursors and direct transport via ocean currents (Butt et al., 2010).

PFASs were detected in China as well, including sea mammals in the south china sea (Lam et al., 2016), surface water (Lu et al., 2015), soil (Li et al., 2020), and landfills (Xu et al., 2021). According to a study conducted in Australia, earthworms contained PFOS due to the usage of aqueous film-forming foam (AFFF) (Das et al., 2013).

In the USA, Oliaei et al., (2012) had discovered that a per/polyfluorinated chemical (PFC) production plant contaminated the surrounding area and aquatic animals. A study focused on the surface soil in the USA and around the world found that the presence of PFASs was evident in all sample points (Rankin et al., 2016). Giesy and Kannan (2001) found out that perfluorooctanesulfonate (PFOS) can be detected in multiple wildlife species including fish, birds, and mammals in North American Great Lakes, Baltic Sea, and the Mediterranean Sea. All the studies have indicated that PFASs are a group of contaminants that are present in our surrounding environment.

2.1.3. PFAS exposure and the effects to human body

The use of PFASs in various applications has led to studies demonstrating that PFASs accumulated in animals (Martin et al., 2003a; Martin et al., 2003b; Ahrens et al., 2016; Giesy and Kannan., 2001).

The human body can also inhale or intake PFASs via numerous pathways. Food packages and food can be one of the major pathways for humans to take up PFASs (Domingo et al., 2012; Domingo, 2012; Clarke et al., 2010). Another pathway through which humans may acquire PFASs is through drinking water (Boiteux et al., 2012; Heo et al., 2014; Schwanz et al., 2016). Last but not the least, air pollution and the dust in the air also contaminated with PFASs since PFASs have already been released into the environment, and they can last for many years (Karásková et al., 2016; Ericson Jogsten et al., 2012; Haug et al., 2011; Fraser et al., 2013).

Recent studies have revealed that PFASs are toxic to humans (Kung et al., 2020), endocrine system (Groh et al., 2019), and the surrounding environment (Zheng et al., 2020; Ait Bamai et al., 2020; Wu et al., 2020). Furthermore, some studies are more focused on the biota, for example, fish tissue (Martin et al., 2003), livestock (Death et al., 2021), and insects (Lan et al., 2020).

Some studies found out that PFASs can accumulate in the human body (Poothong et al., 2017; Hansen et al., 2001; Li et al., 2020; De Silva et al., 2021; Suja et al., 2009). Some other studies focused on the negative effects of PFASs. Cui et al., (2020) found that exposure to PFASs negatively affects male reproductive function. Alexander et al., (2003) discovered that people who worked in a perfluorooctanesulphonyl fluoride (POSF) manufacturing facility were more likely to suffer from bladder cancer and increased mortality rates. According to Ait Bamai et al., (2020), PFASs with a

carbon number above eight are related to allergies and infectious diseases. Kung et al., (2020) found that PFASs, in particular PFOS, have a significant impact on children's lung development.

2.2. PFAS remediation methods

2.2.1. Remediation methods on PFASs

Nowadays, many PFAS remediation methods are being developed and applied on various surface waters, landfill leachate and contaminated sites to remove the existing PFASs in these mediums. They can be categorized into three groups: 1) physical treatment (soil wash, biochar, nanofiltration), 2) chemical treatment (plasma reactor, thermal, oxidation) and 3) biotreatment (microbial, phytoremediation) (Lu et al., 2020; Mahinroosta and Senevirathna, 2020).

Lu et al., (2020) mentioned many different PFAS remediation methods and their combined approaches, except for phytoremediation. Some approaches have a high energy demand, low cost-efficiency, and specific operating conditions (Lu et al., 2020). Soriano et al., (2017) used the combination of nanofiltration followed by electrochemical oxidation to remove perfluorohexanoic acid (PFHxA) from industrial process waters. Sørmo et al., (2021) published a paper recently concerning the use of eight different waste timber biochars to successfully reduce the leachate PFAS concentrations from contaminated soils. Kucharzyk et al., (2017) concluded that many conventional treatments were inefficient in removing PFASs.

Phytoremediation is a low-cost, energy-efficient, less harmful, flexible, and effective method to remove pollutions (Salt et al., 1998). Bolan et al., (2021) also pointed out another advantage of phytoremediation which is its low cost of maintenance. However, the greatest disadvantage of phytoremediation is the enormous amount of time needed to remediate the contaminated soil (Bolan et al., 2021).

2.2.2. Phytoremediation and the application for PFASs

Scientists have introduced the concept of phytoremediation to remediate heavy metal contamination since the 1980s (Cunningham et al., 1995). Numerous studies have demonstrated the potential of phytoremediation as a promising remediation method for contaminated soils since the 1990s (Salt et al., 1998). The concept of phytoremediation involves using green plants, plant-related microorganisms, and soil amendments to concentrate, remove, and degrade dangerous and toxic chemicals in order to reduce the harm to the environment (Salt et al., 1998; Cunningham, 1996; Cunningham et al., 1995). Phytoremediation can be divided into the following types: phytoextraction, phytodegradation, rhizofiltration, phytostabilization, and phytovolatilization (Salt et al., 1998).

Recently, several studies have tested the potential of phytoremediation to remove PFASs (Ghisi et al., 2019; Stahl et al., 2009; Zhang et al., 2019). Blaine et al., (2013) used lettuce (*Lactuca sativa*) and tomato (*Lycopersicon lycopersicum*) to test the uptake of PFAAs. They found that PFBA and PFPeA are taken up best in both lettuce and tomato. Felizeter et al., (2012) focused on growing lettuce (*Lactuca sativa*) on PFAS spiked with different nutrient solutions to test the phytoremediation potential. According to their findings, long-chain PFAAs have higher root concentration factors (RCF) than foliage to root concentration factors (FRCF) while short-chain PFAAs have the opposite findings. As a result, long-chain PFAAs are more likely to stay in the roots while short-chain PFAAs are likely to be stored in foliage. Gobelius et al., (2017) investigated the potential of using phytoremediation to remediate PFASs. In the study, seven plants have been sampled and analyzed. The result shows that leaves and twigs have the highest PFAS concentration among all selected plants. Plants with high root biomass might not be a good phytoextraction

candidate but can be a good option for phytostabilisation. Overall, birch and spruce had the highest PFAS uptake among these seven plant species.

2.2.3. Factors affect the uptake of PFASs

There are few studies focusing on the factors that affect the uptake of PFASs by plants (Blaine et al., 2013; Costello and Lee, 2020).

Stahl et al., (2009) found out that the initial concentration of PFASs in the soil affects the PFAS uptake by the plant. In this article, the higher the concentration of applied perfluorooctanoic acid (PFOA) to the soil, the larger the concentration of PFASs that were absorbed and stored in the plants.

Some studies focused on hydrophobicity and lipophobicity (Felizeter et al., 2012; Xie et al., 2021; Krippner et al., 2014; Zhang et al., 2019). By calculating and comparing the root concentration factors (RCF) and foliage/root concentration factors (FRCF), Felizeter et al., (2012) concluded that long-chain PFAAs tend to be stored in the root due to the long-chain PFAAs' hydrophobicity behavior. A study conducted by Xie et al., (2021) showed a similar result. They pointed out that long-chain PFCAs have a strong hydrophobicity that leads to less mobility. Thus, long-chain PFCAs are less likely to be transported to the shoot, which results in more long-chain PFCAs staying in the roots and soil. As the carbon number in PFASs increases, the property of PFASs becomes more lipophilic (Krippner et al., 2014). Zhang et al., (2019) showed that when the number of carbon atoms increases in the PFAS carbon chain length, the uptake amount decreases. Zhang et al., (2019) also concluded that PFAAs with shorter carbon-chain are more hydrophilic and have smaller K_{ow} . Calderón-Preciado et al., (2012) stated that PFASs with a lower molecular weight have a higher uptake potential. In other words, short-chain PFASs have a higher uptake potential.

Felizeter et al., (2014) discovered that uptake of PFASs by plants is not only controlled by the carbon chain length but by a combination of the chain length and the functional group. PFCAs have a better uptake rate compared to PFSAs since plants show a tendency to absorb the carboxylic group rather than the other functional group (Ghisi et al., 2019; Felizeter et al., 2012; Gredelj et al., 2020). One of the reasons is that plants uptake PFSAs and PFCAs by different pathways (Gredelj et al., 2020).

Plant characteristics can also affect the PFAS uptake. Plants with the following characteristics are considered more suitable for phytoremediation: special uptake capabilities in the root, the shoot has the ability of translocation of the pollutants, accumulation and degradation of the pollutants (Baudh et al., 2017). The plants which are most suitable for phytoremediation are hyperaccumulators and have a high biomass production (Rodriguez et al., 2005).

One major effect on the uptake of the contaminants is the evapotranspiration rate from the plants. Manzoor et al., (2018) mentioned that evapotranspiration is a process that is responsible for absorbing water, nutrients, and contaminants from a growing media into the plant and transport to the shoot. However, this process can be influenced by many factors such as precipitation, irrigation, percolation, and changes in soil moisture (Raja and Bishnoi, 1990).

The root growth can affect the plant to take up the PFASs. Gredelj et al., (2020) discovered that higher root surface in the soil increases the absorption of PFAS by plants. One factor that affects the root growth is the application of fertilizer, however, the additional fertilizer can also affect the PFAS uptake directly. Higgins and Luthy, (2006) found out that with increasing concentration of Ca^{2+} in the soil, the sorption of PFASs to the soil increased. They also conclude that compared to PFAS sorption to minerals, the sorption of PFASs is more driven by organic matter. One method that can increase the soil organic matter is to increase the microbe community. Kallenbach et al., (2016) stated that the microbe community not only stable the soil organic matter (SOM) but also can accumulate SOM. Campos Pereira et al., (2018) found out that PFSAs can have a stronger absorption with soil organic matter (SOM) than PFCAs. Long-chain PFASs bind more strongly with the SOM compared to short-chain PFASs.

The soil organic carbon (SOC) concentration can also affect the plant uptake of PFASs. PFASs have the tendency to stick to SOC (Blaine et al., 2014; Milinovic et al., 2015; Lasee et al., 2020). As a result, it is difficult for plants to take up PFASs if the SOC concentration is relatively high.

2.2.4. Plant species

Plants with hyperaccumulating ability and toxic tolerant properties are considered to be potential plants for phytoremediation (Bauddh et al., 2017). Additionally, using potential hyperaccumulator plants for phytoremediation can not only clean up the contamination but can also result in efficient land use (Bauddh et al., 2017).

Since PFASs are hard to degrade, phytodegradation might not be a good option to deal with PFAS pollution since phytodegradation is mostly used to degrade organic pollutants (Bauddh et al., 2017). On the other hand, phytoextraction can efficiently remediate heavy metal pollution (Bauddh et al., 2017), which might have the potential to extract PFASs from the soil to the plant's tissue.

Mustard, hemp, and sunflower are all hyperaccumulator plants and also have a certain degree of toxic tolerance (Bauddh et al., 2017). The following paragraph showed the previous study which applied phytoremediation to deal with heavy metal pollutions with these plants.

Shi and Cai, (2009) and Shi et al., (2012) tested the tolerance of hemp on cadmium and found out hemp has the ability to accumulate the cadmium. Ahmad et al., (2016) not only tested the phytoremediation potential on cadmium but also the six other heavy metals from industrial emissions. The result showed that copper (Cu), cadmium (Cd), and nickel (Ni) can be accumulated in hemp. A recent study by Stonehouse et al., (2020) used hemp to remediate the Selenium (Se) contamination in the USA and confirmed hemp can also be one of the candidates for the remediation of Se pollution. Ximenez-Embun et al. (2001) states that sunflower is effective in removing lead (Pb), chromium (Cr), zinc (Zn), Cd, and Ni from water. Jadia and Fulekar, (2008) also tested the absorption of heavy metals by sunflower under the influence of amended soil. The result showed that the increase of the biomass of the sunflower plant helps to uptake more heavy metals. Rizwan et al., (2016) also confirmed that with the amendments applied to the sunflower plants, the uptake of heavy metals will increase. Mustard is a kind of plant used a lot in the phytoremediation of heavy metals. They have been well studied for the uptake of Cd (Rizwan et al., 2018; Qadir et al., 2004; Goswami and Das, 2015), Pb (Gurajala et al., 2019), aluminum (Al) (Ahmad et al., 2018) and Zn (Singh and Fulekar, 2012).

Since mustard, hemp, and sunflower are all hyperaccumulator plants (Bauddh et al., 2017), this study aims to test their phytoextraction potential on PFAS. There are few papers focused on using mustard, hemp, and sunflower to remediate PFAS pollution, however, several articles already investigate the plant uptake of PFASs (Bizkarguenaga et al., 2016; Blaine et al., 2013; Blaine et al., 2014; Calderón-Preciado et al., 2012; Felizeter et al., 2012; Felizeter et al., 2014; Ghisi et al., 2019; Gobelius et al., 2017; Gredelj et al., 2020; Krippner et al., 2014; Wen et al., 2014; Xiang et al., 2018; Zhang et al., 2019; Zhao et al., 2017).

According to the findings above, this study investigated whether these three plants would have the same tendency to uptake PFASs as they would uptake the heavy metal pollutions

3. Material and Methods

3.1. Regents and materials

In this study, 12 PFASs were analysed including: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDODA, PFHxS, PFOS. The detailed information can be found in Table A1 in the Appendix. An internal standard (IS) was used for internal calibration, see Table A2 (Franke et al., 2019).

Methanol (99.9% hyper grade for LC-MS, LiChrosolv®, Merck, Germany) and Millipore (Millipak® Express 20, 0.22µm filter, Merck Millipore) were used for the sample cleaning and ultrahigh performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) analysis. Acetonitrile (99.9% hyper grade for LC-MS, LiChrosolv®, Merck, Germany) was used for the solid-phase extraction (SPE) in this study.

Whatman™ Glass Microfiber Filters GF/C™ (47 mm diameter, 1.2 µm) was used for water sample filtering. Oasis HLB 6 cc Vac Cartridge (200 mg Sorbent per Cartridge, 30 µm) was used during solid-phase extraction.

There are two different clean-up cartridges which were used after the SPE depending on the sample types. The Supelclean SPE Tubes (Supelclean™ ENVI-Carb™ SPE Tubes bed wt. 1 g, volume 12 mL) were used for plant and soil samples. The other Oasis cartridges (Oasis HLB 6 cc Vac Cartridge, 200 mg Sorbent per Cartridge, 30 µm) were used for water samples (Rehrl et al., 2020).

The fertilizer used in this experiment is provided by Wallco Plant Nutrition 51 10 43+ micro fertilizer. The content of the fertilizer is shown in Table 1.

Table 1. Content of Wallco Växtnäring fertilizer

Wallco Växtnäring 51 10 43 + micro fertilizer (g of nutrients L ⁻¹)	
Nitrogen (N)	51
Ammonium	20
Nitrate	31
Phosphorus (P)	10
Potassium (K)	43
Sulfur (S)	4
Calcium (Ca)	3
Magnesium (Mg)	4
Iron (Fe)	0.17
Manganese (Mn)	0.2
Boron (B)	0.1
Zinc (Zn)	0.03
Copper (Cu)	0.015
Molybdenum (Mo)	0.0004

The microbe fertilizer used in this experiment is from Tarantula Beneficial Bacteria Liquid, for the content see Table 2 and the original content is shown in Figure A 1.

Table 2. Content of Tarantula Beneficial Bacteria Liquid fertilizer.

Tarantula Beneficial Bacteria Liquid (colony-forming unit (cfu) mL ⁻¹)	
<i>Arthrobacter globiformis</i>	25,000
<i>Bacillus brevis</i>	1,000,000
<i>Bacillus coagulans</i>	500,000
<i>Bacillus licheniformis</i>	5,000,000
<i>Bacillus megaterium</i>	500,000
<i>Bacillus polymyxa</i>	50,000
<i>Bacillus pumilus</i>	50,000
<i>Bacillus subtilis</i>	1,000,000
<i>Bacillus thuringiensis</i>	100,000
<i>Bacillus thuringiensis canadiensis</i>	50,000
<i>Paenibacillus polymyxa</i>	300,000

The soil in this study is S-jord garden soil (provided by the Hasselfors company) and the content of the soil is indicated in Table 3 and the original information is shown in Figure A 2.

Table 3. Content of S-jord garden soil.

S-jord garden soil	
Composition	Sifted light peat, black peat, perlite, Sand, lime
Additives	Limestone flour, dolomite flour
Grain size	Fine grain
pH	5.5~6.5
Nutrients (g m ⁻³)	
Nitrogen (N)	125
Phosphorus (P)	65
Potassium (K)	135
Magnesium (Mg)	225
Calcium (Ca)	1,800
Sulfur (S)	70
Boron (B)	0.3
Copper (Cu)	1.1
Iron (Fe)	1.0
Manganese (Mn)	1.5
Molybdenum (Mo)	0.5
Zinc (Zn)	0.4

3.2. Greenhouse pot culture experiment

3.2.1. Plant species

In this study, mustard (*Brassica juncea*), sunflower (*Helianthus annuus*), and industrial hemp (*Cannabis sativa*) were selected as the subjects in the study.

3.2.2. Preparation of the spiked soil and water treatments

A PFAS mix (Table. A1) was prepared to achieve a concentration of 1 µg g⁻¹ soil for each individual PFASs. First, small portions of the soil (1 kg) were spiked with a PFAS mix and shaken for 1 week in an overhead shaker (Heidolph Reax 2 overhead shaker, Germany) to obtain a homogenized mixture in the form of sludge. The sludge was then manually mixed using a shovel with the rest of the soil (37 kg) by adding 1 kg of unspiked soil to the mixture soil until all the unspiked soil was added to the soil mixture to obtain a homogeneous soil mixture with a theoretical concentration 1 µg of each individual PFASs per gram of soil. The mixed soil was distributed into pots (n = 36) so that every pot had 1 kg wet weight (ww) of the soil and left to rest for a day. Finally, the soil was aged for two weeks in darkness at 4 degrees in the fridge before the plantation in order to meet the equilibrant state.

Four treatments were studied in this experiment: i) microbes, ii) fertilizer, iii) fertilizer plus microbes and iv) tap water as control (no fertilizer and no microbes) (Table 4). i) The microbes were mixed with water of a ratio 1 L water plus 2 mL Tarantula Beneficial Bacteria Liquid (Table 2). ii) The fertilizer water was made in the greenhouse, which is located at the Biocenter at SLU (Table 1). iii) The fertilizer water plus microbes were mixed with fertilizer water of a ratio 1 L water plus 2 mL Tarantula Beneficial Bacteria Liquid. iv) Regular tap water from SLU, Uppsala was used. These treatments were applied throughout the experiment to irrigate the plants. Both the tap water and

fertilizer water were provided by the greenhouse. For each treatment, 5 mL was applied to each pot in treatment i-iv twice per week in the beginning to 200 mL twice per day before harvested.

Table 4. Content of different treatment water for each plant type.

Treatment 1 (<i>n</i> = 3)	Treatment 2 (<i>n</i> = 3)	Treatment 3 (<i>n</i> = 3)	Treatment 4 (<i>n</i> = 3)
Tap water	Tap water with microbe fertilizer (Tarantula Beneficial Bacteria Liquid)	Fertilizer water (Wallco Plant Nutrition)	Fertilizer water (Wallco Plant Nutrition) with microbe fertilizer (Tarantula Beneficial Bacteria Liquid)

3.2.3. Greenhouse pot culture conditions

All plants (i.e., mustard, sunflower, and industrial hemp) were pregrown in small pots with unspiked soil for 6 weeks. The reasons behind this is that 1.) we observed a plant illness due to high concentration of contamination in the previous intership task and 2.) the experiment design according to literature review (Bizkarguenaga et al., 2016; Felizeter et al., 2012; Gredelj et al., 2020; Zhang et al., 2019). Next, the plants were transplanted into 3 L pot (13.7 cm (width) *13.7 cm (length) *23 cm (high)) with 1 kg spiked PFAS soil and the pots were placed in the greenhouse, which is located at the Biocenter at SLU. One plant was placed in each pot. In total, 12 pots of each plant were prepared and triplicate was included for each treatment (i-iv).

The environmental conditions in the greenhouse; the temperature is set to 22°C during the day and 18°C during the night, the light/dark is set at 16 hours/8 hours, the intensity of light is about 150 micromoles and humidity is about 50-60%.

3.2.4. Sampling

After three months, the plants were ready for harvest. The plant samples were weighed (wet weight, ww) and classified into root, stem, leaf, and seed categories. Seeds were collected separately only for sunflowers as these can produce oil.

For cleaning the plant samples, firstly the samples were washed three times with Millipore water, then the samples were cleaned twice with a solution of 50:50 methanol: Millipore water solution. The clean samples were frozen overnight at -20°C in the freezer then freeze-dried for 72~96 hours depending on the plant biomass. The dried samples were weighed to obtain the dry weight and ground, then the samples were ready for further solid-phase extraction.

Water samples were collected at the beginning of the experiment and stored in the fridge at 5°C.

The soil samples were collected at two different times. The first soil samples were collected after the spiked soil was made to know the original PFAS concentration. The second soil samples were collected after harvesting the plant samples to determine the remaining PFAS concentration in the soil. Triplicate soil samples were collected both times in each pot for analysis.

3.3. Extraction

Each soil and plant samples were weighed 2 g and placed into a 50 mL PP-tube (tube 1). 50 μL IS of $0.05\ \mu\text{g mL}^{-1}$ concentration was added to all samples for the internal check. All samples were dried for 30 minutes before 7.5 mL and 2 mL of acetonitrile were added to the plant samples and to the soil samples respectively. Samples were shaken by ultrasonicator for 30 minutes and centrifuged for 20 minutes under 3600 rpm. Collecting the supernatant into a 15 mL PP-tube (tube 2) and the procedure was repeated twice by adding 3 mL and 2 mL of acetonitrile to the plant and soil samples respectively (Ahrens et al., 2016; Dalahmeh et al., 2018).

The water samples were first filtered then extracted by the solid phase extraction (SPE) according to the (ISO/DIS 25101:2009) method using Oasis WAX cartridges (Ahrens et al., 2015). Before the SPE, water samples were spiked with 100 μm of the IS mixture ($0.05\ \text{ng mL}^{-1}$). The cartridges were preconditioned with the following liquid: 4 mL 0.1% ammonium hydroxide in methanol, 4 mL methanol, and 4 mL of 2 lillipore water. Adjusting the speed to one drop per-second by using the vacuum and stop cock. A washing process was performed by applying 4 mL of 25 mM ammonium acetate buffer to the cartridges after the samples were filtered, then the cartridges were dried with a centrifuge at 3000 rpm for 2 minutes. The samples were eluted into 15 mL pp-tubes with 4 mL methanol and 8 mL 0.5% ammonium hydroxide in methanol and dried under vacuum. The samples were concentrated using nitrogen gas to 1 mL and the samples were transferred to a 1 mL glass vial. The PP-tube was rinsed three times with methanol and the methanol was transferred to a 1 mL glass vial to ensure the remaining PFASs were collected. The final sample amount was concentrated to exactly 0.5 mL. Triplicate was performed in this study and two solvent blanks were added in between 15 samples.

3.4. Instrumental analysis with LC-MS/MS

All samples were analyzed by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) (Thermo Scientific, Waltham, MA, USA). The analysis was done at the Milkyway laboratory, Department of Aquatic Sciences and Assessment, SLU (for details see Ahrens et al. (2015)). Samples from HPLC-MS/MS were evaluated using Tracefinder version 4.1 and using Microsoft Office Excel for Mac (Version 16.49).

3.5. Quality Control

Negative blanks

Negative blanks ensured that there was no contamination for the extraction water samples. It followed the same procedure as the other samples with the only difference that negative blanks were not spiked with PFASs.

The concentrations in the negative blanks among all PFASs were: PFBA $0.105\ \mu\text{g/mL}$, PFPeA $0.013\ \mu\text{g/mL}$, PFHxA $0.007\ \mu\text{g/mL}$, PFHpA $0.007\ \mu\text{g/mL}$, PFOA $0.004\ \mu\text{g/mL}$, PFNA $0.002\ \mu\text{g/mL}$, PFDA $0.002\ \mu\text{g/mL}$, PFUnDA $0.003\ \mu\text{g/mL}$, PFDoDA $0.003\ \mu\text{g/mL}$, PFBS $0.004\ \mu\text{g/mL}$, PFHxS $0.002\ \mu\text{g/mL}$, PFOS $0.001\ \mu\text{g/mL}$.

Method Detection Limit (MDL) and Method Quantification Limit (MQL)

In total, 8 method blanks and two soil blanks were added in the analysis and determined the background noise. The method detection limit and method quantification limit were then calculated by the blanks shown below:

$$MDL = \left(\frac{\text{concentration of samples}}{\frac{\text{signal}}{\text{noise}}} \right) * 3$$
$$MQL = \left(\frac{\text{concentration of samples}}{\frac{\text{signal}}{\text{noise}}} \right) * 10$$

where signal and noise are quantified from Tracefinder version 4. For more information (MDL, MQL and IS recovery) see Table A 3 and Table A 4.

3.6. Data Analysis

The following factors will be calculated in excel and discussed in this thesis: Root Concentration Factor (RCF), Shoot Concentration Factor (SCF), Bioconcentration Factor (BCF), and Translocation Factor (TF). The equations are shown below:

$$RCF = \frac{Concentration(root)}{Concentration(soil)}$$

$$SCF = \frac{Concentration(shoot)}{Concentration(soil)}$$

$$BCF = \frac{Concentration(plant)}{Concentration(soil)}$$

$$TF = \frac{Concentration(shoot)}{Concentration(root)}$$

where concentration(root), concentration(shoot), concentration(plant), and concentration(soil) are the PFAS concentration of root, shoot, whole plant, and the PFAS concentration remains in the soil ($\mu\text{g g}^{-1}$ dry weight), respectively. The concentration of shoot is the mixture of concentration of seed, the concentration of leaves, the concentration of stem.

An ANOVA test was performed by R with normalized data from the experiment to test the significant between each factor and will present in the result and discussion section. The reason why using ANOVA is because we want to know which factors will make an impact on the experiment.

4. Results and Discussion

4.1. PFASs in soil before the treatment

The majority of PFASs in the soil before the plants' growth is different with the soil treated by plants (Figure 1), with around 70% of PFCAs and 30% of PFSA. The distribution of PFSA increases from 30% in the soil before treatment to more than 60% after the soil has been remediated. The reason for this might be the plants took up more PFCAs than PFSA. As a result, the PFSA distribution was higher in the soil after plant growth compared to the soil state before the treatment. Among all PFSA, the distribution proportion of PFOS increased the most from 18% in the soil before treatment to 40% after the hemp treatment.

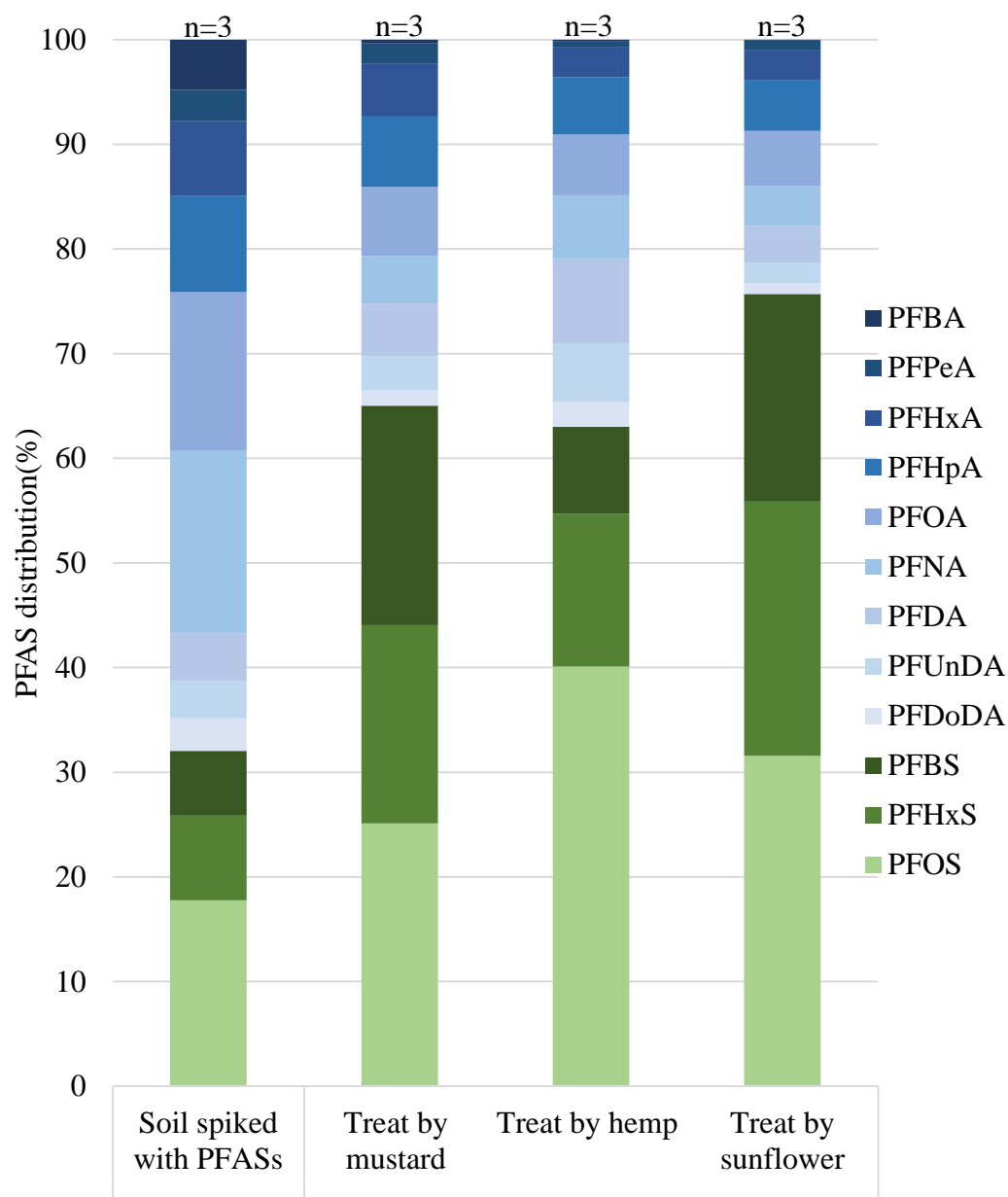


Figure 1. PFAS distribution proportion in different soil samples before and after 40 days of treatment by mustard, hemp, and sunflower.

4.2. PFAS in Plants

4.2.1. Sunflower

Figure 2 shows the concentration and distribution of PFASs in the different compartments of sunflower. Shoots (seed, leaf, and stem) have a total 1.0 µg/g dw of sum PFASs with 74% PFCAs

and 26 % PFASs of sum PFASs. Leaves have the highest sum PFAS concentration (0.78 $\mu\text{g/g dw}$), followed by stem (0.16 $\mu\text{g/g dw}$), seed (0.09 $\mu\text{g/g dw}$), root (0.06 $\mu\text{g/g dw}$). Dominant PFASs were short-chain PFASs, such as PFBA, PFPeA, and PFBS, in leaves with a concentration of 0.21, 0.13, and 0.15 $\mu\text{g/g dw}$. The sum of PFAS concentration in the stem (0.16 $\mu\text{g/g dw}$), roots (0.06 $\mu\text{g/g dw}$), and seeds (0.09 $\mu\text{g/g dw}$) are all smaller than 0.2 $\mu\text{g/g dw}$. In the stem and the seeds, the majority of the PFAS concentration were PFCAs with 96% and 90% of the sum PFASs. The roots have a higher PFASs (28% of sum PFASs) concentration than the stem (4% of sum PFASs) and seeds (10% of sum PFASs).

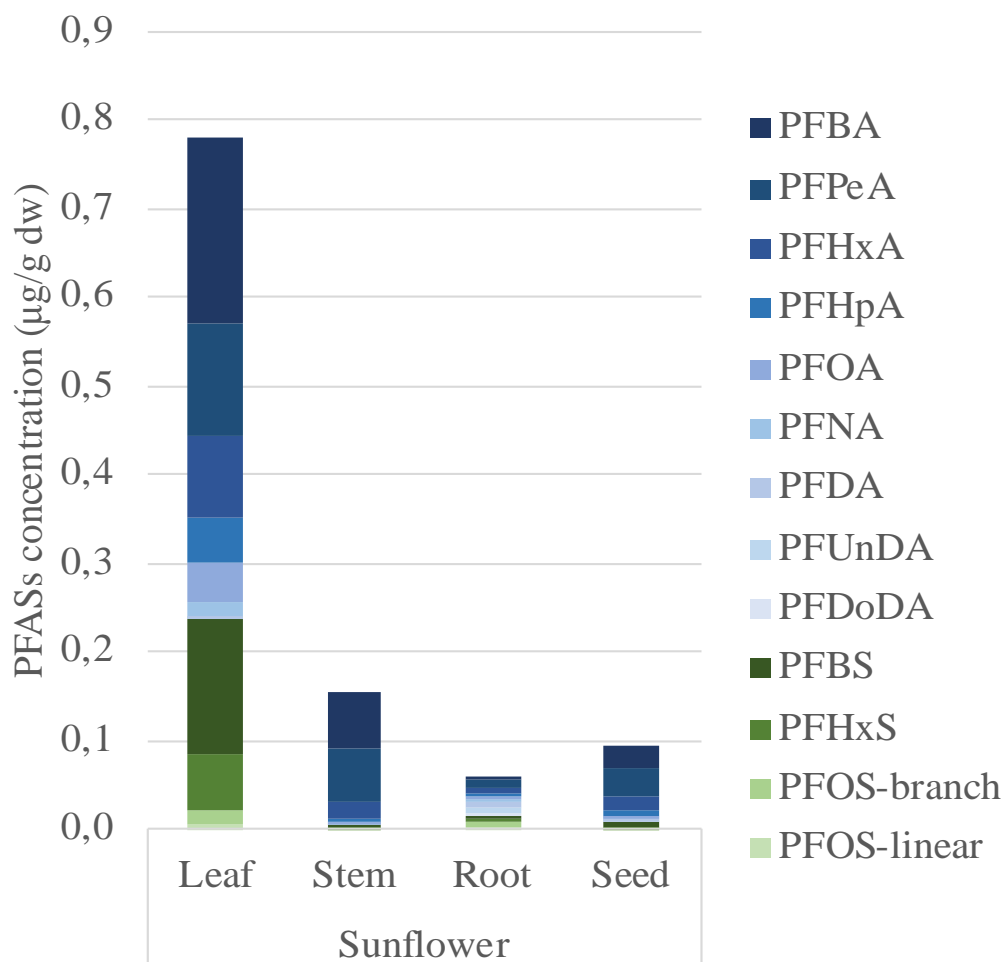


Figure 2. PFAS concentrations in different sunflower compartments ($n = 3$ for each tissue type) irrigated by tap water.

Short-chain PFASs (i.e., PFBA and PFBS) and PFCAs (i.e., PFBA, PFPeA, PFHxA, and PFHpA) account for more than 70% of total PFAS uptake in sunflower (Figure A4). The uptake for PFBA and PFPeA in the whole plant was 34% and 28%, respectively, followed by PFHxA and PFBA, both 11%. This already considered the weight of the different tissue types (total burden). However, long-chain PFASs do not show a similar result, for example, the uptake ratio for both PFUnDA (0.2%) and PFDoDA (0.1%) were less than 1 %, which means sunflower has the tendency to take up short-chain PFASs (i.e., PFBA and PFBS) but less long-chain PFASs (i.e., PFUnDA and PFDoDA). Taking into account that PFCAs represented more than 80% of total PFASs in the plant showing that PFCAs are better taken up compared to PFASs by sunflower.

4.2.2. Hemp

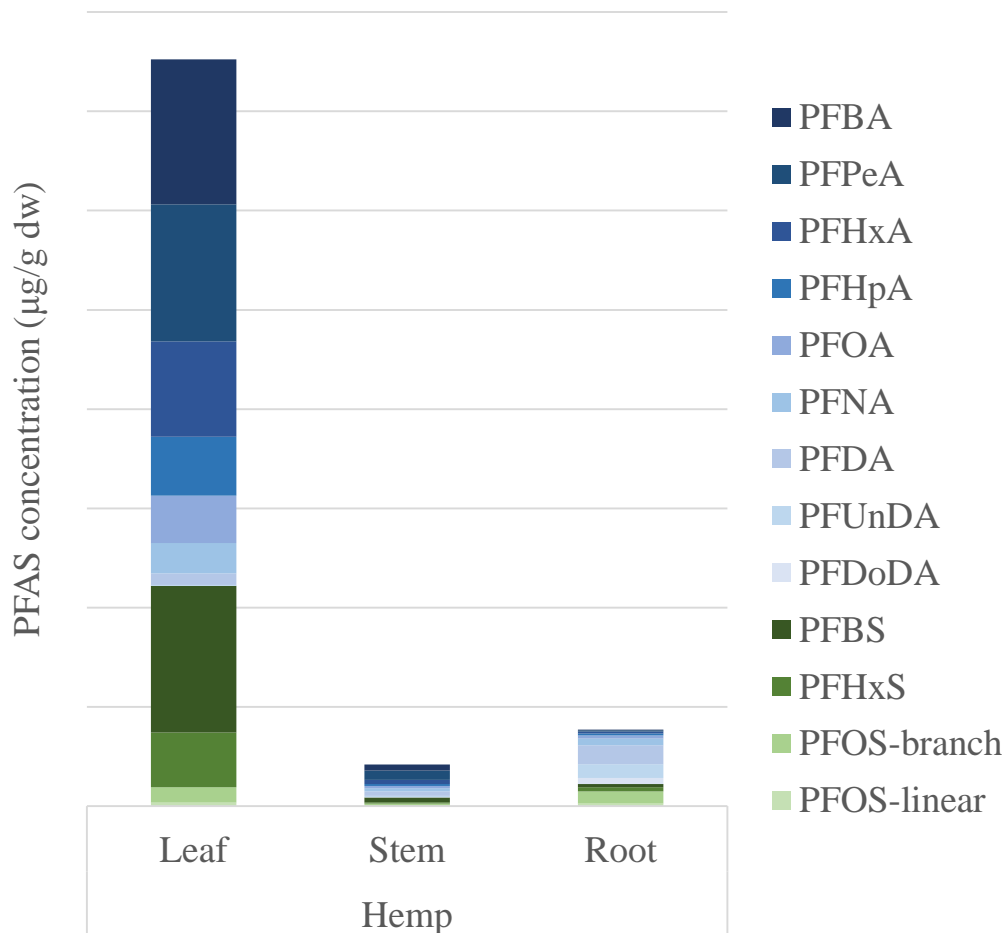


Figure 3. PFAS concentrations in different hemp compartments ($n = 3$ for each tissue type) irrigated by tap water.

Hemp has a similar trend as sunflower with a higher PFAS concentration in the shoot (leaf and stem) rather than in the roots (Figure 3). The leaves have a total $0.75 \mu\text{g/g dw}$ of PFASs. PFBA, PFPeA, and PFBS have the highest concentrations in the leaves with concentrations of 0.15 , 0.14 , and $0.15 \mu\text{g/g dw}$, respectively. Although PFAS concentration in stem ($0.04 \mu\text{g/g dw}$) and root ($0.08 \mu\text{g/g dw}$) are all smaller than $0.1 \mu\text{g/g dw}$, the majority group of PFASs in these two compartments are different. Short-chain PFASs (i.e., PFBA and PFPeA) accounted for the majority in stem while the most PFASs in the roots are long-chain PFASs (i.e., PFDA, PFUnDA, and PFOS).

The distribution of PFASs for hemp is shown in Figure A 3. On the one hand, short-chain PFASs, i.e., PFBA (19%), PFPeA (19%), and PFBS (19%) are the majority of the total uptake. Followed by PFHxA with 13%. On the other hand, the uptake proportion for long-chain PFASs is smaller than 1% (i.e., PFUnDA 0.6% and PFDoDA 0.1%). Overall, the proportion of PFASs and PFCA in hemp is about 3:7.

4.2.3. Mustard

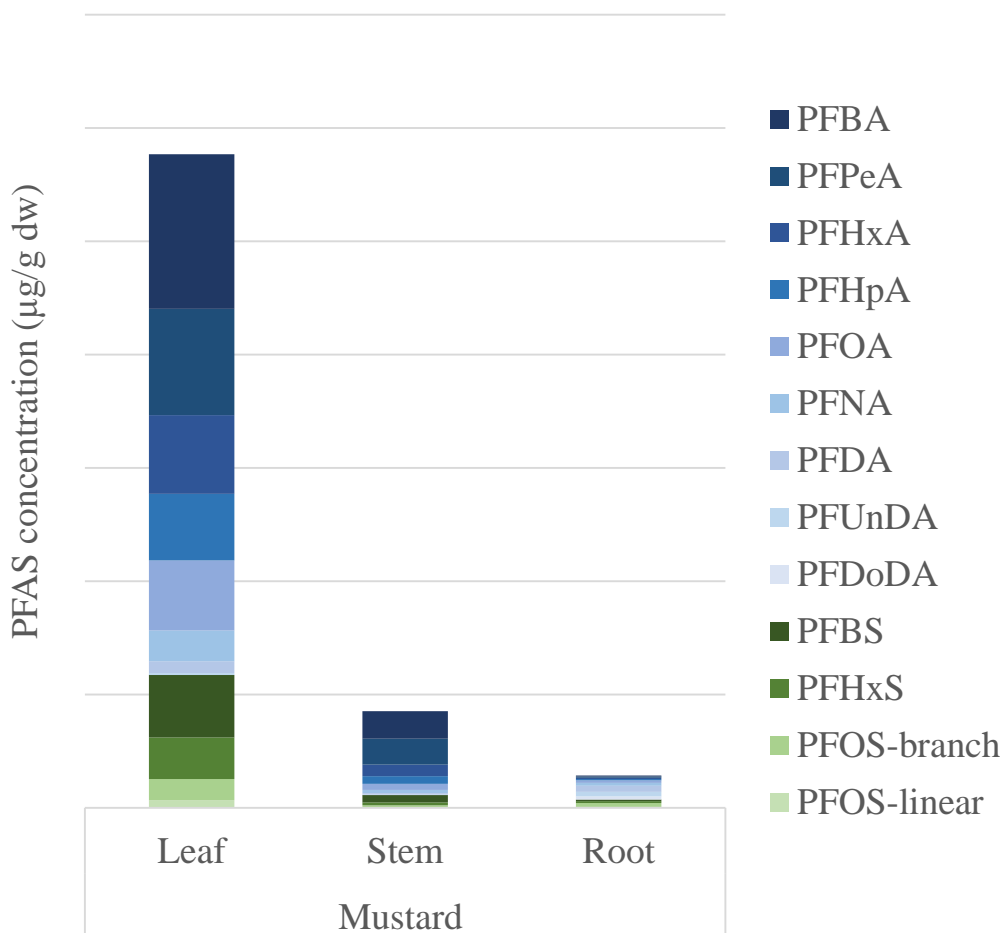


Figure 4. PFAS concentrations in different mustard compartments ($n = 3$ for each tissue type) irrigated by tap water.

The leaves have the highest PFAS concentration with a total of $1.15 \mu\text{g/g dw}$ in mustard shown in Figure 4, while the stem has a concentration of $0.17 \mu\text{g/g dw}$ and the roots have a concentration of $0.06 \mu\text{g/g dw}$. PFAS concentration decreases while the carbon chain length increases in leaves, i.e., from PFBA ($0.27 \mu\text{g/g dw}$) to PFDoDA (not detectable). PFBA has the highest concentration in leaves with a concentration of $0.27 \mu\text{g/g dw}$, followed by PFPeA with $0.19 \mu\text{g/g dw}$. Total PFAS concentration in the stem ($0.17 \mu\text{g/g dw}$) and in the roots ($0.06 \mu\text{g/g dw}$) are all smaller than $0.1 \mu\text{g/g dw}$. The proportion for long-chain PFASs (i.e., PFDA and PFUnDA) in root is higher than 50%, however, for the stem, the majority PFAS group is short-chain PFASs (i.e., PFBA and PFPeA).

Figure A 3 shows in mustard, the most PFASs is PFBA, which is 25%. The second is PFPeA with 19%. PFHxA, PFHpA, and PFBS is 12%, 9%, and 9%, respectively. Short-chain PFASs account for more than 50% of the total uptake.

Although the absolute concentrations of different kinds of PFASs are different in different references, there are some similar trends that can be observed. Some literature has a similar trend that the shorter the carbon chain of PFASs, the higher the PFAS concentration in the shoots, which is similar to what we found in all three plants (Krippner et al., 2014; Bizkarguenaga et al., 2016; Blaine et al., 2013; Felizeter et al., 2012; Felizeter et al., 2014; Gredelj et al., 2020; Wen et al.,

2014). On the other hand, long-chain PFASs (i.e., PHUnDA and PFDoDA) in our result showed higher concentration in root when the carbon chain increased, this result can also be found in other literature (Felizeter et al., 2012; Felizeter et al., 2014; Gredelj et al., 2020),

4.3. Total plant burden

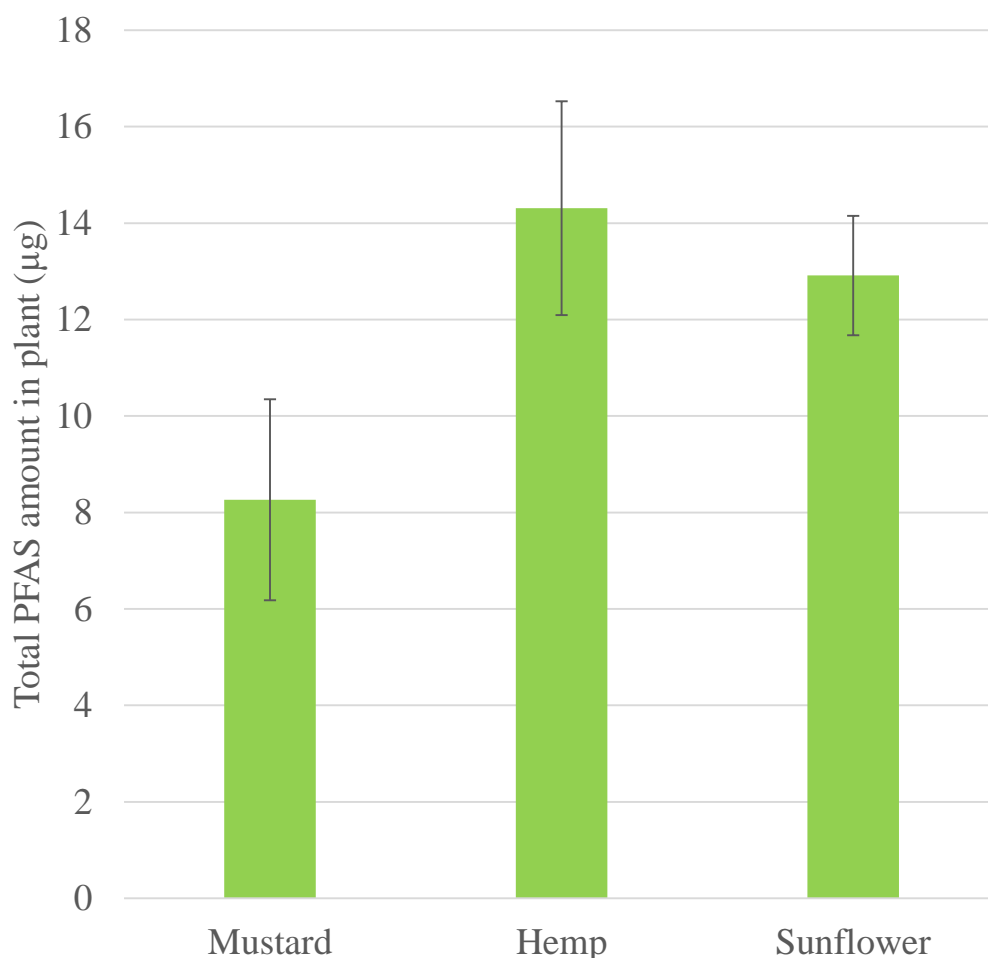


Figure 5. Total PFAS burden in control in mustard, hemp, and sunflower ($n = 3$ for each plant).

The reason for comparing total plant burden is because phytoremediation uses the whole plant to remove the contaminants. The total PFAS amount in hemp, which is 14 µg on average, is slightly higher than the amount of PFASs in sunflower, which is 13 µg on average, see Figure 5. The amount of PFASs in mustard, which is 8.3 µg on average, is about 60% of the PFAS uptake in both hemp and sunflower. The reason might be that the biomass for the hemp (47.1g/plant dw) and the sunflower (54.4g/plant dw) is about double the biomass of the mustard (23.8g/plant dw). Another reason can be the plant evapotranspiration rates; however, evapotranspiration rates can be affected by many different factors, i.e., local weather conditions and cropping system such as plant species, planting date (Penman, 1948). There were some articles already investigating the evapotranspiration rates for mustard, hemp, and sunflower. The evapotranspiration rates for hemp (on average, 470 mm) and sunflower (on average, 439 mm) are much higher than the one for mustard (on average, 320 mm) (Echarte et al., 2019; Pejić et al., 2018; Sharma and Singh, 1993).

4.4. Effect of fertilized water and microbes

The following section will discuss the effect of different amendments on PFAS uptake with normalized data.

Figure 6 and Figure 7 are showing how the treatments affect the uptake of PFASs. It is important to note that PFAS concentration in mustard roots amended with microbes is not included because of problems with the analysis. After adding the fertilizer water to all three plants, a clear increase in biomass can be observed for mustard (on average, 11% increase), industrial Hemp (on average, 29% increase), and sunflower (on average, 1% increase). The total PFAS concentrations in the shoots were lower in the treatment with fertilizer water (on average, 0.67 μg for mustard, 0.48 μg for hemp, and 0.56 μg for sunflower) and microbes plus fertilizer water (on average, 0.87 μg for mustard, 0.4 μg for hemp, and 0.49 μg for sunflower) compared to the control with tap water (on average, 1.32 μg for mustard, 0.79 μg for hemp, and 1.0 μg for sunflower). This indicates that the enhanced plant growth due to the addition amendment could have resulted in a dilution of the PFAS concentration. Another explanation could be that the fertilizer decreases the uptake of PFASs due to the increased interactions of PFASs in the soil by the added ions (Higgins and Luthy, 2006).

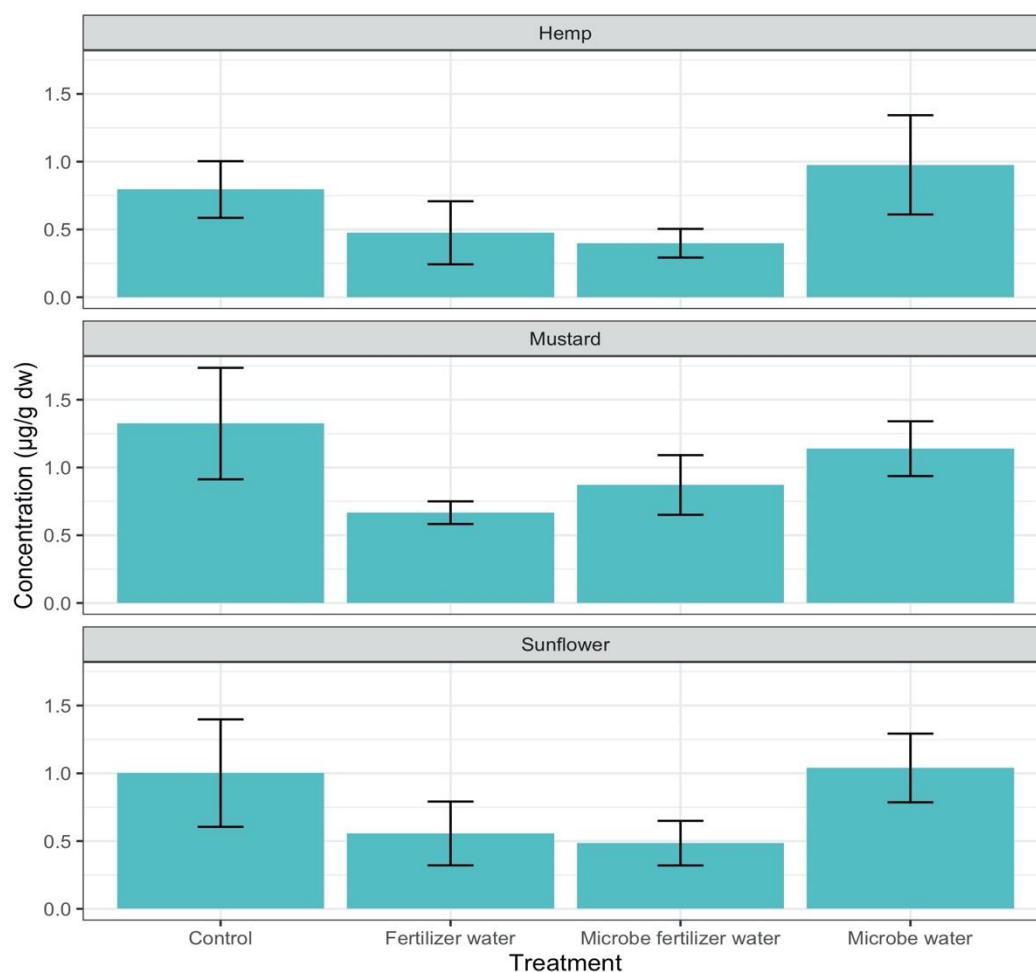


Figure 6. PFAS concentrations in the shoot for the different treatment options ($n = 3$ for each plant).

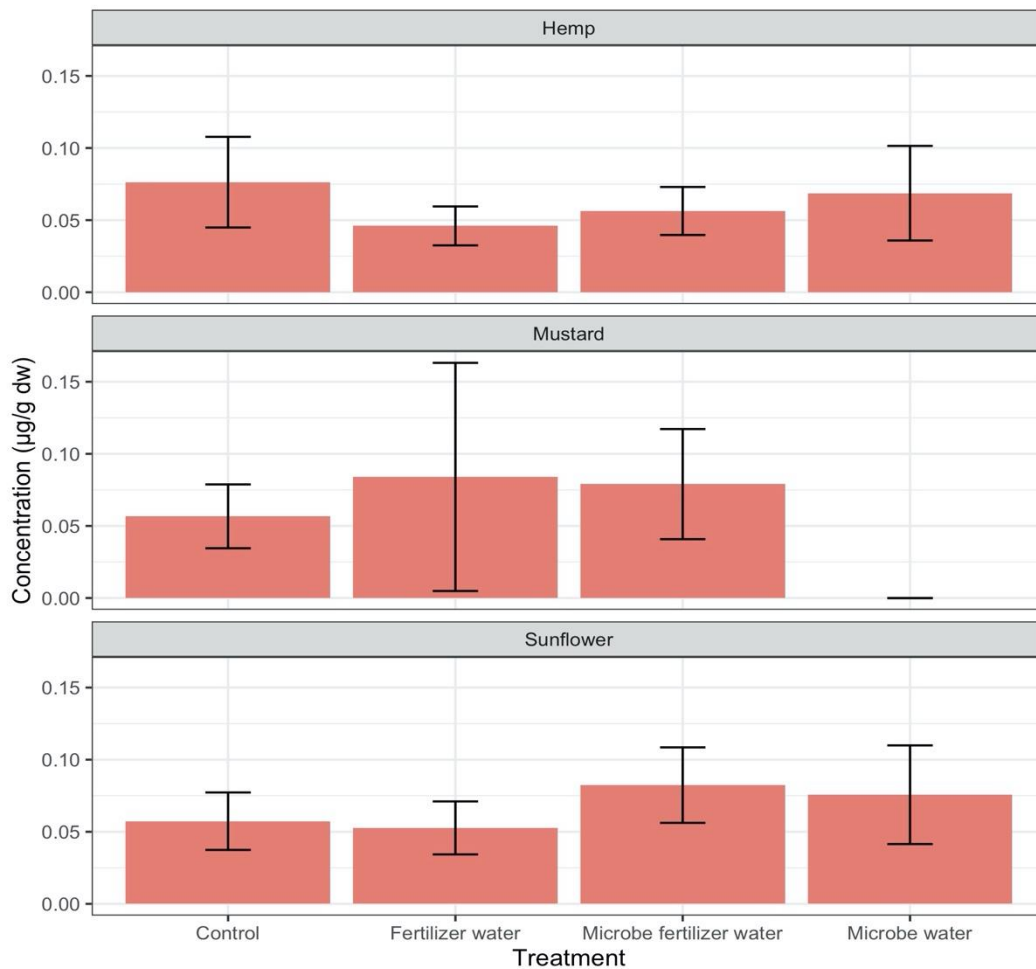


Figure 7. PFAS concentrations in the root for the different treatment options ($n = 3$ for each plant). It is important to note that PFAS concentration in mustard roots amended with microbes is not included because of problems with the analysis.

With the application of microbes plus fertilizer water, the concentration of PFASs in hemp (on average, $0.4 \mu\text{g}/\text{plant dw}$) and sunflower shoots (on average, $0.49 \mu\text{g}/\text{plant dw}$) increased while the concentration in mustard (on average, $0.87 \mu\text{g}/\text{plant dw}$) decreased compared to using only fertilizer water (on average, $0.67 \mu\text{g}/\text{plant dw}$ for mustard, $0.48 \mu\text{g}/\text{plant dw}$ for hemp, and $0.49 \mu\text{g}/\text{plant dw}$ for sunflower).

An ANOVA test was done to study whether the factors were significant (Table 5). The ANOVA used the normalisation data. Three factors are considered in the ANOVA model, which are treatments, plant species, and compartments. The treatment factor considers four different treatments into ANOVA model: Control, Fertilizer water, Microbe water, and Microbe fertilizer water. The plant species factor considers three different plants into ANOVA model: mustard, sunflower, and hemp. The compartment factor considers five different compartments into ANOVA model: seed, leaf, stem, root, and soil. Residuals are the remaining undescribed variances that can affect the result. In the result, all three factors are significant at the significance level set at 0.05 which means these three factors can affect the result (PFAS concentration) of the experiment.

Table 5. ANOVA test (significant codes: $<0.0001 = ***$).

	Df	Sum Sq	Mean Sq	F Value	P-value
Treatment	3	0.522	0.174	8.696	$2.49 \times 10^{-5} ***$
Plant species	2	0.570	0.285	14.243	$2.34 \times 10^{-6} ***$
Compartment	4	9.167	2.292	114.437	$<2 \times 10^{-16} ***$
Residuals	140	2.804	0.020		

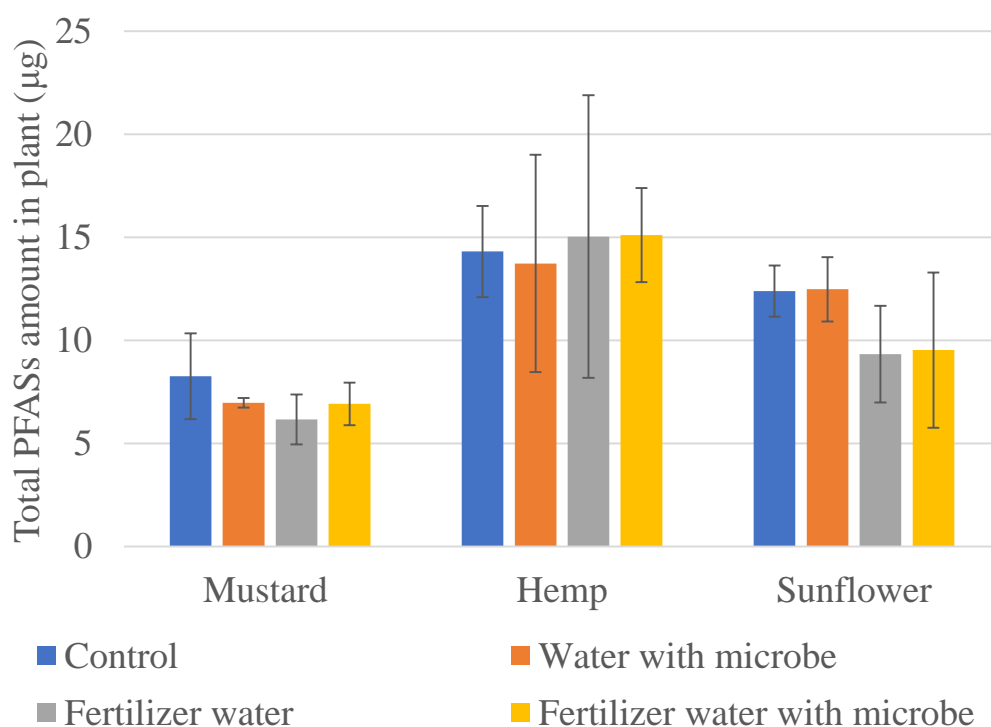


Figure 8. PFAS amounts in different treatments for mustard, industrial hemp, and sunflower ($n = 3$ for each treatment).

The reason for comparing total plant burden is because phytoremediation uses the whole plant to remove the contaminants. In Figure 8, the uptake of the PFASs in each plant combined with different treatments were between 5 µg and 15 µg on average. Hemp had the highest average PFAS uptake (14.5 µg). Hemp treated with fertilizer water had the highest standard deviation (6.9 µg). The additional fertilizer treatment and fertilizer water with microbe treatment increased the PFAS uptake in hemp by 0.7 µg and 0.8 µg, respectively, compared to the control sample. A decreased PFAS uptake happened when only the microbe water was applied to the hemp (13.7 µg). If we want to have a better statistical power, then it will require an up-scale field experiment since the physiological difference between plants may influence the PFAS absorption.

Sunflower with the control (12.4 µg) and microbe water treatment (12.5 µg) showed higher PFAS uptake compared with the other two treatments (9.3 µg for fertilizer water and 9.5 µg for microbes plus fertilizer water). After adding the fertilizer water, average PFAS uptake by sunflower decreased by about 3 µg. However, fertilizer water with microbe treatment had a larger standard deviation (3.8 µg) which can result in a higher PFAS uptake among all treatments.

Mustard had the lowest average PFAS uptake (7.1 µg) of the three selected plants with less than 10 µg. One of the reasons for this is because the biomass for mustard (23.8g dw) is much smaller than hemp (47.1g dw) and sunflower (54.4g dw). Interestingly, after adding different fertilizer treatments, the uptake of PFASs declined in all three treatments for mustard (from 8.3 µg to 6.16 µg).

Different treatments can influence different kinds of plants differently. Fertilizer increased PFAS uptake by the hemp but reduced the PFAS uptake for mustard and sunflower. Water with microbe did lower the PFAS uptake in mustard and hemp but enhanced sunflower to take up more PFASs, this might be because of the PFASs binding with the microbe instead of being absorbed by the plant (Zhao et al., 2014).

4.5. Different analysis factors

Root concentration factor (RCF) describes the absorption of chemicals into the roots (Briggs, Bromilow and Evans, 1982). Shoot concentration factor describes the tendency of chemicals to be stored in the shoot (Wu et al., 2012). Translocation factor (TF) is an important factor that can access the ability of plants to transport the chemicals from the roots to shoot, which is an indication of potential phytoremediation purpose (Trotta et al., 2006). Bioconcentration factor is a factor that tells the ability of a plant to accumulate chemicals from the media or substrate (Pandey and Baudh, 2019).

In Table 6, all three plants have a higher RCF value for PFCAs than PFASs. In Hemp, there is a clear decline in the RCF value for all PFASs when amendments have been applied to Hemp. Mustard has the lowest RCF value compared to the other two plants. Mustard and sunflower have a better RCF value for PFBA compared to Hemp. Sunflower has an overall good RCF value of the three plants especially for the short-chain PFCAs and also for the PFDoDA. However, comparing Table 6 and Table 7, there is clear evidence that although short-chain PFASs have higher RCF values, their SCF values are 10 times or even 100 times higher, which means these short-chain PFASs tend to be stored in the shoot, which can refer to TF value. In long-chain PFASs, the story is totally opposite. RCF values for long-chain PFASs in Table 6 are obviously larger than the SCF values in Table 7, which means the long-chain PFASs are more willing to stay in the roots.

Similar RCFs patterns for the PFCAs have been observed in Wen et al., (2014). The PFBA and PFDoDA had a higher value while the PFOA, PFNA, and PFDA had a lower RCF value. Zhang et al., (2020) and Felizeter et al., (2014) also found that long-chain PFASs had a higher RCF value. The reason for this might be because the transportation of short-chain PFCAs is driven by transpiration (Zhao et al., 2017). Another reason might be that sorption to the roots or lipid-rich root solids (Felizeter et al., 2012) since long-chain PFASs have hydrophobic characteristics (Zhang et al., 2019).

In Table 7, for both PFCAs and PFASs, as the carbon chain number increases, the value of SCF decreases. SCF values range from 3051 to 0, which means shorter chain PFASs tend to be transported to the shoot while long-chain PFASs tend to stay in the roots. For hemp, control, irrigated with the combination of water, and microbe fertilizer, SCF values for PFHpA, PFOA, PFNA, and PFHxS are higher than other combinations. Sunflower irrigated with fertilizer water has overall higher SCF values compared to other treatments except for PFBA and PFBS. The highest SCF value is 3051 and it is the combination of the sunflower irrigated by tap water with microbes and PFBA. Hemp and sunflower have an outstanding SCF value for both PFBA and PFPeA in comparison to mustard.

Similar patterns were shown in Wen et al., (2014), where the carbon chain length and SCF were negatively correlated. Felizeter et al., (2014) had similar results of the SCF for the short-chain PFASs. In their study, the SCF, leaf concentration factor and edible part concentration factors have been calculated and discovered that the short-chain PFASs were transported to the edible part of the plant while the long-chain PFASs showed no tendency to be transported to the shoot. In our study, we compared both RCF and SCF for PFOA, PFNA, PFDA, and PFOS; these compounds might have strong sorption to the stem tissue, which was also shown in Felizeter et al., (2014).

Zhao et al., (2017) and Xiang et al., (2018) focused on the uptake of PFOA. Both articles found out that the RCF value of PFOA is higher than the SCF value. However, in our result, PFOA had higher values on SCF in three plants rather than RCF, which might be a better phytoremediation selection plant since PFOA will mostly transport to shoot and be harvested.

TF value can range from 2628 to 0 in Table 8. There is a decrease trend in TF value when the carbon-chain length increases, which means long-chain PFASs tend to stay in root while the short-chain

PFASs tend to stay in shoot (this can refer back to SCF and RCF). Compared with other plants, hemp has better TF value among all selected PFAS, followed by mustard, then sunflower. After adding amendments to the plants, TF value for short carbon-chain PFASs increases in hemp and sunflower but not mustard. This means hemp and sunflower have better phytoextraction effects on short-chain PFASs. One thing to consider is that the TF values for short-chain PFCA (Table 8) is one or two orders more than the TF values for short-chain PFCA, which means the tendency for short-chain PFCA to transport to shoot is greater than them being absorbed in the roots.

The BCF value can range from 3218 to 0.2 and as shown in Table 9. The longer the carbon chain length in PFASs, the smaller the BCF value. Hemp and sunflower have better BCF value performance than mustard. The additional amendments do not really affect the BCF value for mustard and sunflower, however, sunflower irrigated with fertilizer water has the highest BCF value 3218 for PFBA. Hemp has a higher BCF value when it has been irrigated by tap water and fertilizer water except for PFBS. On one hand, short-chain PFASs such as PFBA, PFPeA, and PFBS have a better BCF value when the hemp been irrigated by fertilizer water. On the other hand, when the number of carbons in the carbon chain is greater than 7, the BCF values are smaller than 10 except for hemp irrigated with water, hemp irrigated with microbe water and sunflower irrigated with fertilizer water.

The accumulation of PFASs declines while the carbon chain number increases, and these results can be found in other articles (Blaine et al., 2013; Bizkarguenaga et al., 2016). One of the reasons for this might be the longer the PFASs carbon chain, the heavier molecular weight it becomes. The absorption from the roots and transpiration through the plants both involve penetrating the membrane (Zhao et al., 2014). This conclusion can also be found in Calderón-Preciado et al., (2012).

Table 6. Root concentration factor (RCF) in mustard, industrial hemp, and sunflower for the different treatments

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDODA	PFBS	PFHxS- branch&liner	PFOS- branch	PFOS- liner
Mustard -Water	10.1	2.3	0.8	0.6	1.2	2.1	4.1	4.7	7.0	0.3	0.3	0.7	0.5
Mustard -Fertilizer water	10.5	5.4	1.3	0.8	1.9	3.2	3.8	4.0	5.2	0.3	0.4	1.0	0.8
Mustard -Fertilizer water with microbe	13.7	4.4	1.3	0.9	1.8	3.2	4.6	5.0	5.9	0.4	0.4	1.2	0.7
Hemp-water	16.5	9.6	2.7	1.3	2.3	4.1	8.0	8.3	8.6	1.5	1.1	1.4	1.0
Hemp-Water with microbe	2.2	3.7	1.6	1.3	1.8	2.9	5.4	5.6	6.5	0.8	0.8	1.1	0.6
Hemp-Fertilizer water	1.5	0.7	0.6	0.4	0.8	1.8	3.2	3.3	3.3	0.7	0.4	1.0	0.6
Hemp-Fertilizer water with microbe	0.6	0.5	0.6	0.6	1.0	2.4	4.0	4.9	6.5	0.6	0.5	1.1	0.6
Sunflower-Water	34.3	23.2	6.5	1.0	1.4	2.3	4.7	7.2	10.0	0.4	0.3	0.9	0.5
Sunflower-Water with microbe	67.8	24.0	5.8	1.5	2.1	3.2	5.1	6.7	9.6	1.2	0.7	1.6	1.0
Sunflower-Fertilizer water	44.1	14.4	5.2	2.5	2.5	2.7	3.3	4.1	5.9	1.2	1.0	0.9	0.7
Sunflower-Fertilizer water with microbe	18.0	12.1	8.4	4.5	4.0	4.1	4.7	9.0	15.7	1.9	1.1	0.9	0.6

(red: between 10 and 100, orange: between 5 and 10, yellow: between 1 and 5, light green: between 0.5 and 1, green: between 0.5 and 0.1)

Table 7. Shoot concentration factor (SCF) in different kinds of plants with different treatments.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDODA	PFBS	PFHxS- branch&liner	PFOS- branch	PFOS- liner
Mustard -Water	527.8	79.8	18.3	10.7	10.8	7.1	2.6	0.8	0.2	3.3	2.2	1.2	1.1
Mustard -Water with microbe	619.6	113.6	23.5	11.4	9.9	5.1	1.9	0.5	0.1	4.4	2.3	1.1	1.0
Mustard -Fertilizer water	412.5	73.8	18.8	10.3	9.7	4.7	1.4	0.4	0.1	2.3	1.8	0.8	0.8
Mustard -Fertilizer water with microbe	347.5	71.6	14.7	9.0	7.6	3.5	1.0	0.3	0.1	2.6	2.0	0.7	0.7
Hemp-water	2122.9	391.4	58.3	18.3	13.9	9.2	3.6	0.8	0.2	30.3	6.2	1.0	0.6
Hemp-Water with microbe	1646.8	512.4	102.0	28.8	16.8	6.9	2.5	0.5	0.1	48.5	6.7	0.8	0.4
Hemp-Fertilizer water	2975.3	431.2	42.2	8.5	3.0	2.3	1.0	0.2	0.0	240.9	4.5	0.6	0.3
Hemp-Fertilizer water with microbe	1691.6	269.4	36.1	8.8	3.1	2.0	0.9	0.3	0.1	134.0	3.7	0.4	0.2
Sunflower-Water	1946.8	139.1	18.7	5.7	3.6	2.1	1.2	0.5	0.5	3.9	1.1	0.3	0.2
Sunflower-Water with microbe	3051.2	143.2	23.1	6.9	4.4	2.7	1.9	1.0	1.1	5.3	1.6	0.5	0.4
Sunflower-Fertilizer water	2569.6	212.1	44.7	10.7	5.7	2.6	1.3	0.7	0.8	3.1	1.8	0.4	0.4
Sunflower-Fertilizer water with microbe	1646.9	166.1	24.5	6.1	3.6	2.2	1.3	0.8	0.8	2.6	1.1	0.3	0.2

(pink: above 1000, dark orange: between 1000 and 100, red: between 100 and 10, orange: between 10 and 5, yellow: between 5 and 1, light green: between 1 and 0.5, green: between 0.5 and 0.1, blue: smaller than 0.1)

Table 8. Translocation factor (TF) in different kinds of plants with different treatments.

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFBS	PFHxS- branch&liner	PFOS- branch	PFOS- liner
Mustard -Water	52.1	34.6	23.1	16.7	8.7	3.3	0.6	0.2	0.0	12.1	6.6	1.7	2.3
Mustard -Fertilizer water	39.3	13.6	15.0	12.7	5.2	1.5	0.4	0.1	0.0	6.9	5.1	0.8	1.0
Mustard -Fertilizer water with microbe	25.4	16.3	11.7	9.7	4.2	1.1	0.2	0.1	0.0	6.7	4.8	0.6	0.9
Hemp-water	128.4	40.9	21.8	14.2	6.1	2.2	0.5	0.1	0.0	20.4	5.5	0.7	0.6
Hemp-Water with microbe	760.1	137.0	64.0	21.8	9.1	2.4	0.5	0.1	0.0	59.9	8.3	0.7	0.7
Hemp-Fertilizer water	1932.7	598.7	72.5	19.2	3.9	1.3	0.3	0.1	0.0	362.7	10.1	0.6	0.6
Hemp-Fertilizer water with microbe	2628.5	585.4	63.2	14.3	3.1	0.8	0.2	0.1	0.0	210.5	7.1	0.3	0.4
Sunflower-Water	56.8	6.0	2.9	5.6	2.6	0.9	0.2	0.1	0.0	10.1	3.4	0.4	0.5
Sunflower-Water with microbe	45.0	6.0	4.0	4.7	2.1	0.9	0.4	0.1	0.1	4.4	2.3	0.3	0.4
Sunflower-Fertilizer water	58.2	14.8	8.6	4.3	2.3	1.0	0.4	0.2	0.1	2.5	1.9	0.4	0.5
Sunflower-Fertilizer water with microbe	91.6	13.7	2.9	1.3	0.9	0.5	0.3	0.1	0.1	1.4	1.1	0.3	0.4

(pink: above 1000, dark orange: between 1000 and 100, red: between 100 and 10, orange: between 10 and 5, yellow: between 5 and 1, light green: between 1 and 0.5, green: between 0.5 and 0.1, blue: smaller than 0.1)

Table 9. Bioconcentration factor (BCF) in different kinds of plants with different treatments

	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFBS	PFHxS- branch&liner	PFOS- branch	PFOS- liner
Mustard -Water	445.7	67.6	15.5	9.1	9.3	6.3	2.8	1.3	1.2	2.8	1.9	1.1	1.0
Mustard -Fertilizer water	236.0	48.9	10.4	6.7	6.2	3.3	1.6	1.2	1.3	1.5	1.4	0.6	0.6
Mustard -Fertilizer water with microbe	303.3	62.0	12.6	8.1	7.2	3.9	1.9	1.3	1.3	2.4	2.0	1.0	0.8
Hemp-water	2020.8	372.6	55.6	17.4	13.3	9.0	3.9	1.2	0.7	28.9	6.0	1.0	0.7
Hemp-Water with microbe	1493.4	468.2	93.4	26.4	15.4	6.6	2.7	0.8	0.5	44.2	6.2	0.8	0.5
Hemp-Fertilizer water	2798.7	405.7	39.8	8.0	2.8	2.2	1.1	0.4	0.2	226.7	4.3	0.6	0.3
Hemp-Fertilizer water with microbe	1584.6	252.3	33.8	8.2	2.9	2.0	1.1	0.6	0.5	125.5	3.4	0.4	0.2
Sunflower-Water	2525.8	178.0	24.9	8.7	7.1	4.4	2.2	1.2	1.1	4.1	1.4	0.6	0.5
Sunflower-Water with microbe	3218.8	143.2	18.6	6.2	4.7	2.5	1.4	0.9	0.8	4.0	1.3	0.5	0.4
Sunflower-Fertilizer water	3125.8	246.8	40.4	10.0	7.0	2.8	1.2	0.7	0.7	2.7	1.6	0.4	0.4
Sunflower-Fertilizer water with microbe	2189.6	201.4	24.3	7.0	5.3	3.1	1.8	1.8	2.6	2.7	1.3	0.5	0.4

(pink: above 1000, dark orange: between 1000 and 100, red: between 100 and 10, orange: between 10 and 5, yellow: between 5 and 1, light green: between 1 and 0.5, green: between 0.5 and 0.1, blue: smaller than 0.1)

4.6. Phytoremediation potential

In the following, one remediation scenario was performed to test the potential of using hemp to remediate a former firefighting training facility. At the end, the estimated time required was also presented in Table 10.

Sørmo et al., (2021) tested the contaminated soil which was a former firefighting training facility in Norway. According to their result, the most dominant PFASs were PFOS ($1000 \pm 60 \mu\text{g kg}^{-1}$) and PFHxS ($110 \pm 24 \mu\text{g kg}^{-1}$), but they also detected PFBS ($3.9 \pm 0.7 \mu\text{g kg}^{-1}$), PFBA ($2.4 \pm 0.4 \mu\text{g kg}^{-1}$), PFHxA ($8.2 \pm 1.7 \mu\text{g kg}^{-1}$), and PFOA ($6.4 \pm 1.1 \mu\text{g kg}^{-1}$) in the soil. The reason why we selected this site was because the PFASs investigated in Sørmo et al., (2021) have a high overlap with the PFASs in this study.

If the consulting company would like to use phytoremediation to remove the PFASs in soil, based on the result from this experiment, hemp might be selected as it had the highest PFAS uptake. According to the (Hash et al., 2020) guideline, the recommended spacing for planting hemp is 10 to 15 cm apart, in this case, we choose 10 cm to maximum the number of the hemp, ending up with 970,000 hemp/hectare.

Assuming the bulk density is 1.33 g/cm^3 for a medium texture soil according to USDA (Soil Quality Indicators, 2008), 1 hectare of soil with a depth of 1 meter will end up with 13,300,000 kg of soil (assumed land area). Due to the climate condition in Norway, hemp can only be harvested once per year. Using hemp as a phytoremediation method, it was 0.4, 1.0, 2.7, 2.7, 1.8, $0.9 \mu\text{g absolute/year}$ removed of PFOS, PFHxS, PFBS, PFBA, PFHxA, and PFOA, respectively. Using hemp as a phytoremediation method, it would take about 31,905 years, 1548 years, 20 years, 13 years, 64 years, and 100 years to remediate the PFOS, PFHxS, PFBS, PFBA, PFHxA, and PFOA, respectively, in the former firefighting training facility which Sørmo et al., (2021) mentions about (Table 10). The equation performed in the calculation is shown below:

$$\begin{aligned} & \text{Estimated time (year)} \\ &= \frac{\text{concentration of PFASs in the training facility} * \text{soil weight}}{(\text{PFAS remove per year} * \text{number of hems can be planted in assumed land area})} \end{aligned}$$

It seems to be practical for using hemp to remediate the PFBA and PFBS in this site but not for the other PFASs simply because it will take a long time for remediating other PFASs. If it is possible to double the times for harvesting, the time needed for phytoremediation will be even shorter. To conclude, combining different remediation methods is needed if the plants will be applied to phytoremediation practice to reduce the remediation time for all the PFAS contaminants.

Table 10. Phytoremediation at a PFAS-contaminated site in Norway (Sørmo et al., 2021)

PFASs	Soil concentration ($\mu\text{g kg}^{-1}$ of soil)	Total PFAS contamination ($\mu\text{g absolute}$)	Removal of PFASs per year ($\mu\text{g absolute}$)	Estimated time needed for phytoremediation (years)
PFBA	2.4	$1.3 \cdot 10^{10}$	2.7	13
PFHxA	8.2	$1.5 \cdot 10^9$	1.8	64
PFOA	6.4	$5.2 \cdot 10^7$	0.9	100
PFBS	3.9	$3.2 \cdot 10^7$	2.7	20
PFHxS	110	$1.1 \cdot 10^8$	1.0	1548
PFOS	1000	$8.5 \cdot 10^7$	0.4	31,905

4.7. Limitations

There are several limitations in this study.

- Since the soil was manually mixed, there is a possibility of non-homogeneous PFAS distribution in the soil, which results in a different initial PFAS soil condition.
- The plants were transplanted as seedlings into the PFAS spiked soil which could have reduced the phytoextraction period for the plants and reduced the PFAS uptake.
- The size of the pot limited the root growth in depth which could have resulted in less PFAS uptake for the plants.

5. Conclusions

The results from this thesis work confirmed the objectives and hypotheses. Three selected plants were observed to have a tendency to take up and accumulate PFASs, especially for the short chain PFASs. Among all three plants, hemp took up the most PFASs, followed by sunflower and mustard took up the least. The leaves were found to have the highest PFAS amount compared to the other plant compartments. The additional amendments to the plant did not seem to have a big impact on the PFAS uptake. According to the PFAS distribution graph, short-chain PFASs were as expected, absorbed, and stored in the plant. This result is also supported by the TF and BCF values where short-chain PFCA and PFSA were accumulated and transported to the shoot.

When applying phytoremediation, it is important that the fallen leaves are being collected, which has been done in this thesis work, since the leaves stored the most PFASs compared to the other plant compartments.

Further research to increase the efficiency of PFAS uptake of the three plants can be done. Such studies include examining different amendments at different levels to make correlations with PFAS accumulation and combining phytoremediation with other remediation techniques. Microbiome analysis should be done to monitor the difference in microbial diversity during the experiment and their effects on PFAS uptake. Upscaling and conducting of the study at PFAS containment sites to test the phytoremediation potential for these three plants can also be done.

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Appendix 1

Table A 1. Analyzed per- and polyfluoroalkyl substances.

Names	Abbreviation	Molecular formation
Perfluorinated carboxylic acids (PFCAs)		
Perfluorobutanoic acid	PFBA	$C_3F_7CO_2^-$
Perfluoropentanoic acid	PFPeA	$C_4F_9CO_2^-$
Perfluorohexanoic acid	PFHxA	$C_5F_{11}CO_2^-$
Perfluoroheptanoic acid	PFHpA	$C_6F_{13}CO_2^-$
Perfluorooctanoic acid	PFOA	$C_7F_{15}CO_2^-$
Perfluorononanoic acid	PFNA	$C_8F_{17}CO_2^-$
Perfluorodecanoic acid	PFDA	$C_9F_{19}CO_2^-$
Perfluoroundecanoic acid	PFUnDA	$C_{10}F_{21}CO_2^-$
Perfluorododecanoic acid	PFDoDA	$C_{11}F_{23}CO_2^-$
Perfluoroalkane sulfonic acids (PFASs)		
Perfluorobutane sulfonic acids	PFBS	$C_4F_9SO_3^-$
Perfluorohexane sulfonic acid	PFHxS	$C_6F_{13}SO_3^-$
Perfluorooctane sulfonic acids	PFOS	$C_8F_{17}SO_3^-$

Table A 2. PFAS IS with the corresponding PFAS quantification.

Internal Standard	Corresponding PFAS quantification
13C2 -PFHxA	PFBS, PFPeA, PFHxA
13C4 -PFOA	PFHpA, PFOA
13C5 -PFNA	PFNA
13C2 -PFDA	PFDA
13C2 -PFUnDA	PFUnDA
18O2 -PFHxS	PFHxS, 6:2 FTSA
13C4 -PFOS	PFOS, 8:2 FTSA

Table A 3. Quality control for MDLs.

Blanks	Method Blanks			Soil Blanks		
	Millipore water			Soil		
	(n=8)			(n=2)		
	Blank ($\mu\text{g mL}^{-1}$)	MDLs ($\mu\text{g mL}^{-1}$)	QLs ($\mu\text{g mL}^{-1}$)	Blank ($\mu\text{g g}^{-1}$)	MDLs ($\mu\text{g g}^{-1}$)	QLs ($\mu\text{g mL}^{-1}$)
PFBA	0.1051	0.046	0.152	0.0049	0.007	0.024
PFPeA	0.0131	0.001	0.004	0.0003	ND	0.001
PFHxA	0.0068	0.001	0.002	0.0294	0.003	0.012
PFHpA	0.0064	0.001	0.003	0.0362	0.015	0.050
PFOA	0.0044	ND	0.001	0.0129	0.015	0.050
PFNA	0.0016	ND	0.001	0.0046	0.003	0.010
PFDA	0.0018	ND	0.001	0.2303	0.089	0.295
PFUnDA	0.0029	0.001	0.003	0.0110	0.007	0.023
PFDoDA	0.0033	0.001	0.002	0.0348	0.010	0.032
PFBS	0.0037	0.001	0.003	0.0003	ND	0.001
PFHxS	0.0016	0.001	0.002	0.0004	ND	0.001
PFOS-branch	0.0009	ND	0.001	0.0000	ND	ND
PFOS-linear	0.0009	ND	0.001	0.0008	ND	0.001

Table A 4. IS Recovery

Recovery	Plant		Water		Soil	
	(n = 117)		(n = 4)		(n = 36)	
	Mean	\pm SD	Mean	\pm SD	Mean	\pm SD
¹³ C ₂ PFHxA	117	30	113	0.2	137	12
¹³ C ₄ PFOA	124	25	105	0.1	138	11
¹³ C ₅ PFNA	124	22	92	0.1	139	12
¹³ C ₂ PFDA	117	22	66	0.1	125	9
¹³ C ₂ PFUnDA	108	21	36	0.2	121	10
¹⁸ O ₂ PFHxS	125	24	103	0.1	139	10
¹³ C ₄ PFOS	120	17	75	0.1	130	11

Tarantula_003a-5401-L-14INT-
001A



NON ALIMENTARI VEGETALI INGREDIENTI:

Analisi Garantita:

Ingredienti Modificativi:

Ingredienti Attivi:

Organismi del Terreno.....2%

Arthrobacter globiformis 25 000 cfu/mL

Bacillus brevis..... 2 000 000 cfu/mL

Bacillus coagulans..... 500 000 cfu/mL

Bacillus licheniformis..... 5.000 000 cfu/mL

Bacillus megaterium..... 500 000 cfu/mL

Bacillus polymyxa..... 50 000 cfu/mL

Bacillus pumilus 50 000 cfu/mL

Bacillus subtilis..... 1 000 000 cfu/mL

Bacillus thuringiensis..... 100 000 cfu/mL

Bacillus thuringiensis canadiensis..... 50 000 cfu/mL

Paenibacillus polymyxa..... 300 000 cfu/mL

Ingredienti Inerti:

Acqua.....98%

Indicazioni:

Utilizzare 2mL per Litro durante la prima e la seconda settimana della fase di fioritura. Conservare lontano da fonti di luce, tra 10°C(50F) - 20°C(68F).

AGITARE BENE PRIMA DELL'USO

Precauzioni: Non ingerire. Tenere fuori dalla portata dei bambini.

Figure A 1. Content of microbe fertilizer used irrigation water.



Nyhet!
Finns nu i
18 liter

S-jord

Beskrivning och användning

S-jord är en finsiktad, extra luftig blandning avsedd för plantupptragning i små behållare och såldor. Den är tillverkad av en blandning speciellt utsiktad torv och har en tillsats av perlit och sand. Gödslingen är anpassad för sådd och sticklingförökning.

S-jord kan användas till fyllning av de flesta pluggsystemen.

Den ger genom sin porösa struktur ett substrat som också fungerar bra för sticklingförökning.



Varudeklaration

Varutyp: Jordblandning
Kornstorlek: Fin
pH-värde: 5,5 - 6,5

Förpackningsdata
Volym: 18/50 liter

Sammansättning:
Siktad ljus torv, svarttorv, perlit, sand, kalk

Tillsatser:
Kalkstensmjöl, dolomitmjöl
0,9 kg NPK 14-7-15 med mikronäring, mikronäring

Tillsatt mängd i g/m ³ :			
Kväve (N)	125	Bor (B)	0,3
Fosfor (P)	65	Koppar (Cu)	1,1
Kalium (K)	135	Järn (Fe)	1,0
Magnesium (Mg)	225	Mangan (Mn)	1,5
Kalcium (Ca)	1800	Molybden (Mo)	0,5
Svavel (S)	70	Zink (Zn)	0,4

Leveransfakta

Säckvara levereras i 50 l plastsäck av polyeten, lastad på sträckfilmdad helpall, 18 l på halvpall

Artikelnr	EAN-kod	Vikt/säck	Antal/pall	Vikt/pall
2001	7311610020016	15 kg	45 st	675 kg
2011	7311610020115	6 kg	42 st	252 kg

Figure A 2. The content of soil used for plantation.

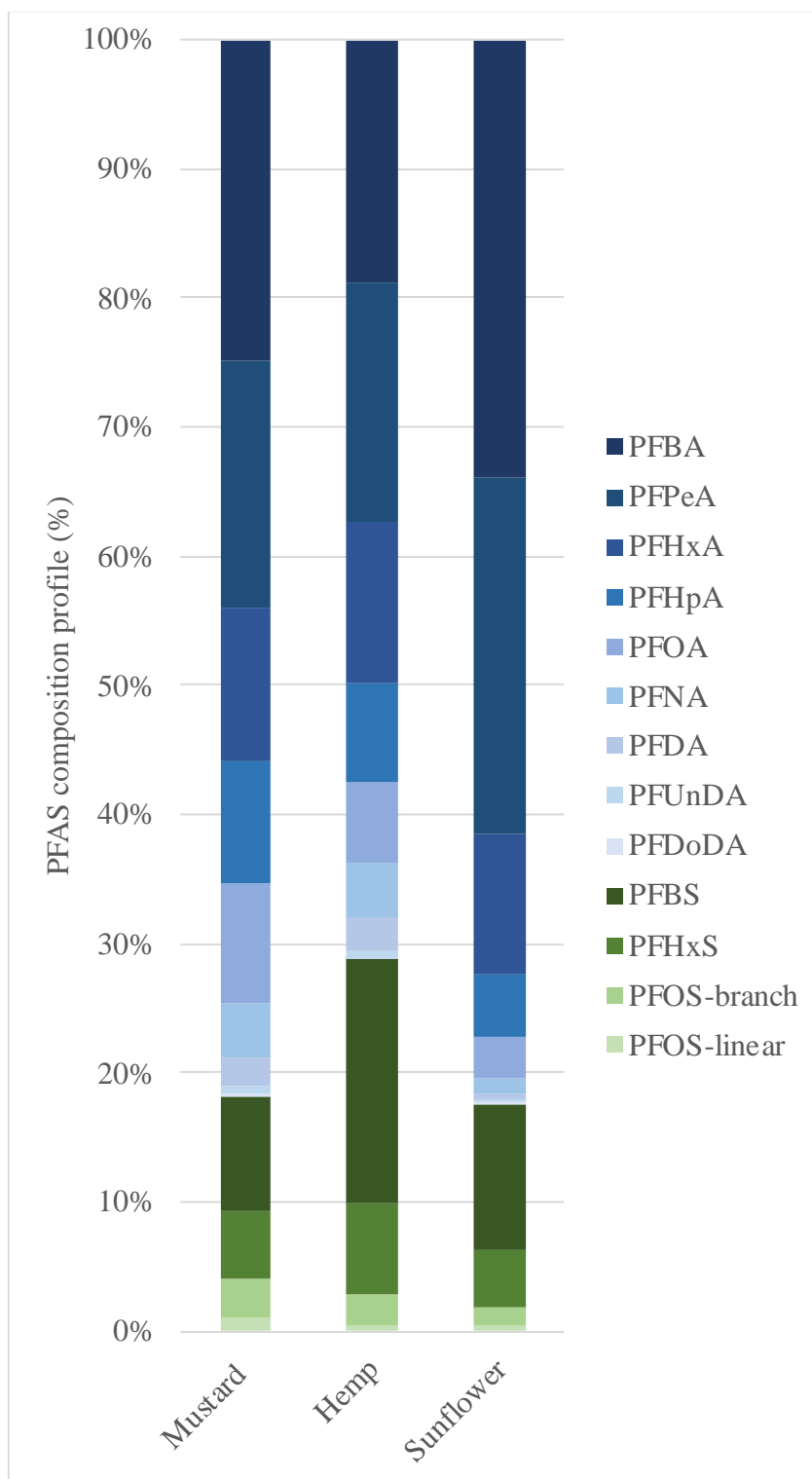


Figure A 3. PFAS distribution in all three plants.